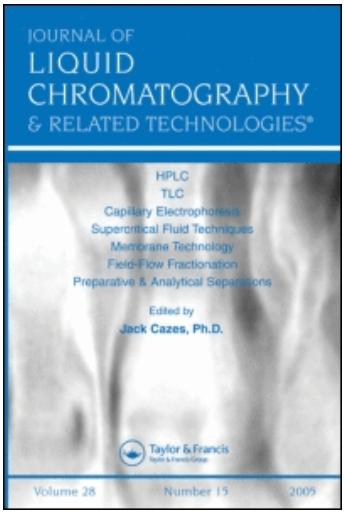

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Quantification of Vigabatrin in Human Plasma by Liquid Chromatography–Electrospray Tandem Mass Spectrometry

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Abstract: A simple, rapid, and accurate liquid chromatographic-mass spectrometric method for quantification of vigabatrin (VGB) in human plasma using 4-phenyl-4-aminobutanoic acid as an internal standard (IS) has been developed and validated. The method involves deproteinization of plasma sample with acetonitrile followed by detection of analytes by tandem mass spectrometry. The drug and the IS were chromatographed using XTerra™ C_{18} column and a mobile phase consisting of acetonitrile/water (50 : 50 v/v) and 0.025% formic acid, at a flow rate of 0.1 mL/min. The effluents were detected in multiple reaction monitoring (MRM) mode using MRM transitions m/z 129.57 > 70.99 and m/z 179.7 > 116.92 for VGB and IS, respectively. The method is significantly fast, as the run-cycle time was <3 min run-to-run. The described method was linear over the VGB concentration range of 0.5–10 μg/mL ($r > 0.999$), with a limit of detection of 0.05 μg/mL. The intra-day coefficient of variation [CV (%)] ranged from 3.84% to 6.53% and the percentage deviation from the nominal value (%DEVs) was in the range of −4.93% to −2.03%. Inter-day CV and %DEVs ranges were 2.78% to 9.15% and −4.91% to 6.67%, respectively. Mean recovery percentage of VGB in spiked human plasma samples was 99.59%. The stability study indicates that VGB in plasma is stable for at least 4 weeks when stored at −20°C. This developed method is significantly

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important in clinical practice for monitoring VGB plasma levels, particularly in pharmacokinetic studies and bioavailability/bioequivalence studies.

Keywords: Vigabatrin, LC-MS/MS, human plasma

INTRODUCTION

Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter that acts on the postsynaptic membranes of CNS to open chloride channels, resulting in membrane hyperpolarization. Following release into the synapse, GABA is degraded by the enzyme GABA-transaminase (GABA-T).[1] Vigabatrin (VGB) [(±)-4-amino-5-hexenoic acid; γ-vinyl-GABA] is a structural analog of the GABA (Fig. 1). It exists as a racemic mixture of *R*(−) and *S*(+) isomers with the latter as the pharmacologically active enantiomer.[2] VGB produces its anticonvulsant effect by irreversibly inhibiting the degradative enzyme GABA-T, leading to an increase in brain levels of GABA.[3] VGB is considered as an effective agent for the management of partial seizures, with or without secondary generalization and infantile spasms.[4] Following oral administration, VGB is rapidly absorbed from the gastro-intestinal tract with peak serum levels being reached within 1 hr. VGB is negligibly bound to plasma proteins and about 70% of the administered dose is excreted unchanged in the urine.[2,3]

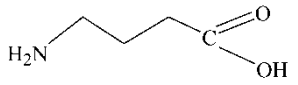
Several analytical techniques for determination of VGB in biological fluids have been reported. Gas chromatographic[5,6] and high-performance liquid chromatographic assays[7–14] using fluorescence detection have been described. The chromatographic assays have several drawbacks such as complex derivatization procedures, lack of sensitivity, and low stability of the measured fluorogenic product. Recently, tandem mass spectrometry (MS/MS) has been considered as a powerful tool for analysis of drugs and biomolecules in biological fluids and has been successfully utilized in many clinical applications such as drug discovery,[15] pharmacokinetics screening of drugs,[16] therapeutic drug monitoring,[17] and detection of drug abuse.[18]

The present study aims at developing a simple, fast, and validated method for the determination of VGB using an LC-MS/MS technique after a single pretreatment step of human plasma sample.

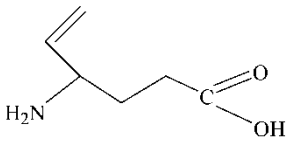
EXPERIMENTAL

Chemicals

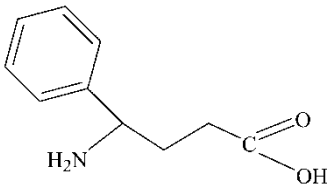
VGB was obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). The internal standard (IS), 4-phenyl-4-aminobutanoic acid, was kindly



Gamma-aminobutyric acid (GABA)



Gamma-Vinyl-GABA (Vigabatrin)



4- phenyl- 4- aminobutanoic acid

Figure 1. Chemical structures of GABA, VGB, and IS (4-phenyl-4-aminobutanoic acid).

provided by Hoechst-Marion-Roussel Inc. (Cincinnati, OH, USA). Acetonitrile was of HPLC grade. Water was purified using a Milli-Q water device (Millipore, Bedford, MA, USA). Human plasma was kindly donated by the central blood bank, MOH, Kuwait. All other chemicals and reagents were of analytical grade.

Instrumentation and Conditions

The chromatographic system, Waters Alliance 2690, consisted of a solvent delivery system and an autosampler (Waters Assoc., Milford, MA, USA). The analytes were chromatographed on an XTerra^{TM} C_{18} column (3 μm,

3 mm i.d. × 50 mm; Waters Assoc., MA, USA) utilizing a mobile phase consisting of acetonitrile/water (50 : 50 v/v) and 0.025% formic acid at a flow rate of 0.1 mL/min. The analyses were conducted at ambient temperature. The eluate was directed into the ionization probe of Triple Quadrupole Mass Spectrometer (Quattro LC, Micromass, Manchester, UK), operated in a positive electrospray ionization mode (+ESI). Tuning parameters for MS and MS/MS scans were optimized by direct infusion of solutions of VGB and the IS in the mobile phase into the ionization chamber at a flow rate of 10 μL/min using a Harvard syringe pump. The ion source and desolvation temperatures were set at 100°C and 250°C, respectively. Capillary voltage was adjusted at 3.2 kV, cone voltage was 20 V, collision energy was 15 eV, and the collision gas pressure was <1.0 mbar. On the basis of MS and MS/MS data, multiple reaction monitoring (MRM) transitions at m/z 129.57 $>$ 70.99 (VGB) and m/z 179.7 $>$ 116.92 (IS) were used for quantification of VGB. Data acquisition and quantitation processes were controlled by MassLynx NT Software (Version 3.5, Micromass).

Standard Solutions and Calibration Curves

Stock solutions (1.0 mg/mL) of VGB and the IS were prepared in acetonitrile/water (50 : 50 v/v). An aliquot of the stock solution was diluted with water to give a VGB working standard solution of 0.2 mg/mL. On the other hand, the IS stock solution (1.0 mg/mL) was further diluted in acetonitrile to yield a working IS solution of 0.2 mg/mL. The calibration curves of VGB were prepared by spiking drug-free human plasma with VGB at concentrations of 0.5, 1, 2, 5, and 10 μg/mL. Similarly, quality control (QC) samples were prepared in drug-free human plasma at concentrations of 0.75, 3.0, and 7.5 μg/mL. The spiked plasma samples (5 mL) were aliquoted (100 μL) into Eppendorff polypropylene tubes and kept frozen at −20°C pending analysis.

Sample Preparation

To 100 μL aliquot of plasma sample was added 500 μL of acetonitrile containing the IS (0.2 mg/mL). The mixture was vortex-mixed for 30 sec and then centrifuged at 13,000 rpm for 10 min. A 100 μL aliquot of the supernatant was transferred to the autosampler and a 20 μL aliquot was injected into the LC-MS/MS system.

Validation Method

The linearity of the developed LC-MS/MS was evaluated by preparing VGB in human plasma at five non-zero calibration standards, over the range of

0.5–10 μg/mL, and then analyzed in replicates of seven. The calibration parameters and data plotting were automatically determined by MassLynx software using weighted linear regression analysis. The lowest limit of quantification (LLOQ) at the coefficient of variation (CV) ≤20% and the lowest limit of detection at (signal : noise ratio of 3 : 1) were determined from the calibration curve. In each calibration batch, QC samples at concentrations of 0.75, 3.0, and 7.5 μg/mL, covering the low, medium, and high ranges of the calibration curve, were analyzed in sets of replicates to assess intra- and inter-day precision and accuracy. The intra-day precision was determined from eight replicate analyses of QC samples from one calibration curve batch in 1 day, whereas the inter-day precision was determined over a period of 4 weeks. The percentage of CV serves as a measure of precision, whereas percentage deviation from the nominal concentrations (%DEVs) is used as a measure of accuracy. The specificity of the method was evaluated by the analysis of six independent sources of the drug-free human plasma. Recoveries of VGB from human plasma were determined by spiking drug-free human plasma (eight replicates for each standard) with known amounts of the drug to achieve VGB concentrations of 0.75, 3.0, and 7.5 μg/mL and analyzing the samples using LC-MS/MS.

Stability of VGB in human plasma was determined through five freeze–thaw cycles (−20 ± 2°C to room temperature). After thawing, samples were allowed to stand on the bench top, under room lighting, until 2 hr had elapsed since their removal from the freezer. Alternatively, the effect of frozen storage on VGB stability in human plasma was assessed through storing of VGB plasma samples at −20°C over a period of 1 month. VGB plasma samples were analyzed immediately after preparation and at selected time intervals after storage over the study period. Stability was defined as <10% loss of initial drug concentration.

RESULTS AND DISCUSSION

As shown in Fig. 1, VGB has a weak chemical functionality that renders its analysis quite difficult. Chemical derivatization was considered for chromatographic analysis of VGB with fluorescence detection. Recently, MS/MS has been extensively used in our laboratories for the analysis of weak UV-absorbing drugs and in neonatal screening. The technique relies on the detection of the compound based on its mass ion rather than chromophoric moiety. For successful quantitation of VGB by LC-MS/MS, the ionization and tuning parameters for detection of the parent and daughter ions of VGB and IS were optimized. Capillary voltage at 3.2 kV, cone voltage at 20 V, and collision energy at 15 eV were selected for optimum detection of parent and daughter ions of VGB and IS. The source and desolvation temperatures were adjusted at 100°C and 250°C, respectively.

A mobile phase consisting of CH_3CN/H_2O in a ratio of 1 : 1 and containing 0.025% formic acid permits maximum formation of mass ions to be detected by positive electrospray ionization. At the earlier-mentioned tuning parameters, the parent ions $[M + H]^+$ of VGB and IS were detected at m/z 129.57 and 179.7, respectively (Figures 2 and 3). These molecular mass ions were further used in MS/MS experiments to determine the daughter ions of the analytes, which were detected at m/z 70.99 and 116.92, respectively. MRM scan was then selected to quantify specifically VGB in plasma samples. As MRM scan relates daughter ion to the parent ion, it is possible to detect VGB and IS independently in mixtures. MRM transitions at m/z $129.57 > 70.99$ and m/z $179.7 > 116.92$ (Figure 4) were selected to

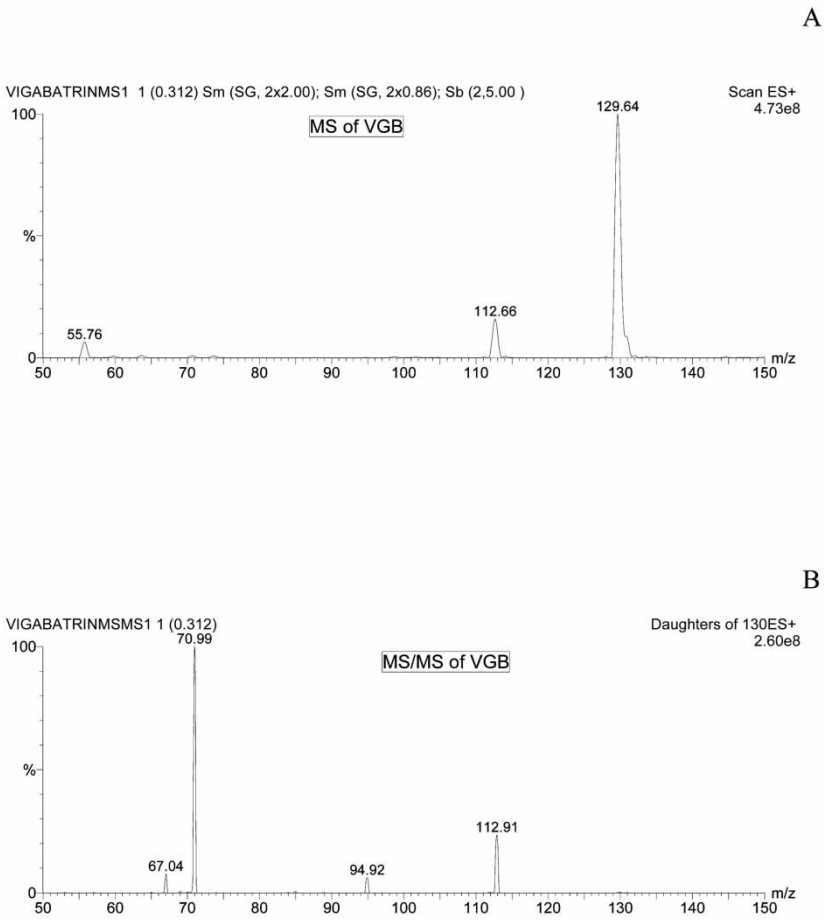


Figure 2. (A) MS and (B) MS/MS spectra of VGB.

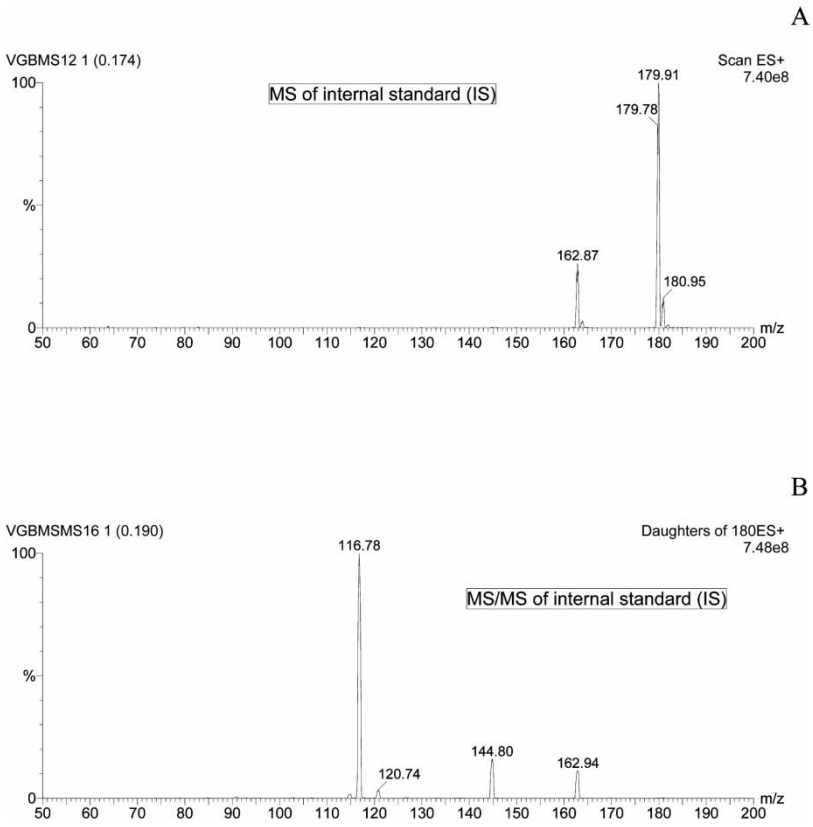


Figure 3. (A) MS and (B) MS/MS spectra of IS.

analyze VGB by the IS method with a high degree of selectivity. Under these conditions, the analytes were rapidly detected (<3 min) without a need of complete chromatographic resolution, as their detection was based on fragmentation properties rather than chromatographic behaviors. Unspiked plasma samples, as blanks, exhibited no interference at the measured MRM transitions of VGB and IS (Figure 5).

Linearity of the developed LC-MS/MS procedure was evaluated by establishing the calibration curves over VGB concentrations in a range of 0.5–10 μg/mL. The selected concentrations were in the range of VGB trough-levels at 1.4–14 μg/mL.[8] The calibration data showed little day-to-day variability of slopes (<10.0%) and correlation coefficients (<0.05%) (Table 1). A typical calibration curve batch indicating linear correlation of peak area ratio of VGB/IS and VGB concentrations was shown in Figure 6. As indicated from the calibration plot, the LLOQ for VGB was 0.5 μg/mL (CV = 7.8%) and the lowest limit of detection was 0.05 μg/mL. The

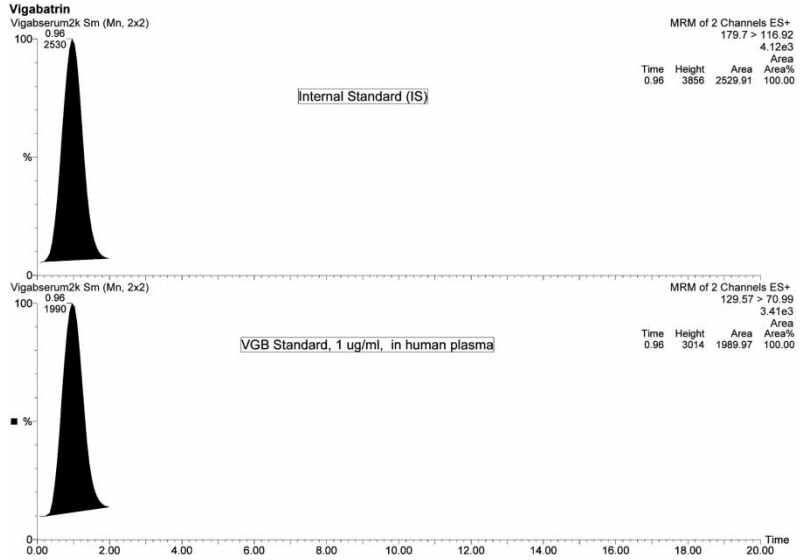


Figure 4. Typical MRM chromatogram of VGB (1.0 μg/mL) and IS in human plasma after protein precipitation with acetonitrile.

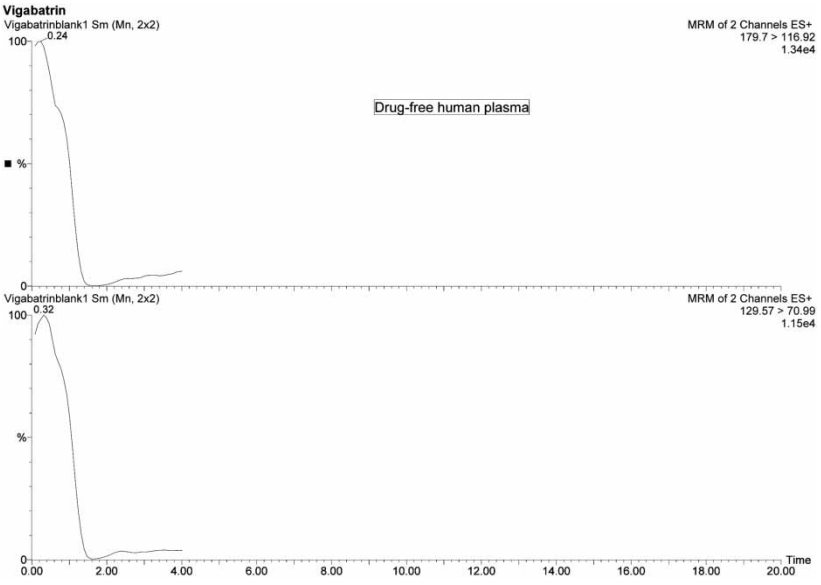


Figure 5. Typical MRM chromatogram of drug-free human plasma.

Table 1. Calibration data for determination of VGB by LC-MS/MS method

	Theoretical concentration (μg/mL)						
Batch no.	0.5	1.0	2.0	5.0	10	Slope	*r*
1	0.353	0.762	1.535	3.790	8.100	0.800	0.998
2	0.415	0.753	1.645	3.641	7.552	0.746	0.999
3	0.333	0.753	1.554	3.867	8.242	0.816	0.999
4	0.401	1.054	1.735	5.005	9.982	1.004	0.999
5	0.341	0.808	1.591	3.788	7.923	0.789	0.999
6	0.387	0.802	1.643	3.863	8.347	0.820	0.998
7	0.362	0.769	1.572	3.809	7.981	0.792	0.999
Mean	0.370	0.814	1.611	3.966	8.304	0.824	0.999
±S.D.	0.029	0.100	0.064	0.430	0.725	0.077	0.0003
CV (%)	7.805	12.281	3.961	10.836	8.730	9.315	0.038

specificity of the developed LC-MS/MS was proven, as the MRM chromatograms of all tested drug-free human plasma samples showed no interference at the selected MRM transitions of VGB and IS (Figure 5). The intra-day precision and accuracy of the developed LC-MS/MS assay was evaluated. The CV (%) and %DEVs were 3.84% to 6.53% and −4.93% to −2.03%, respectively. The inter-day precision and accuracy ranged from 2.78% to 9.15% and from −4.91% to 6.67%, respectively (Table 2). The data indicated high accuracy and reproducibility of the developed method. Recovery studies showed mean percentage recovery of VGB from human plasma in a range of 99.17–100.15% (Table 3) indicating the suitability of protein precipitation in the preparation of samples for analysis.

Stability studies of VGB samples after several freeze–thaw cycles demonstrated that VGB was stable for at least five freeze–thaw runs (Table 4). Alternatively, the data showed that VGB was stable for at least 4 weeks when kept frozen at −20°C, with no appreciable degradation products (Table 5).

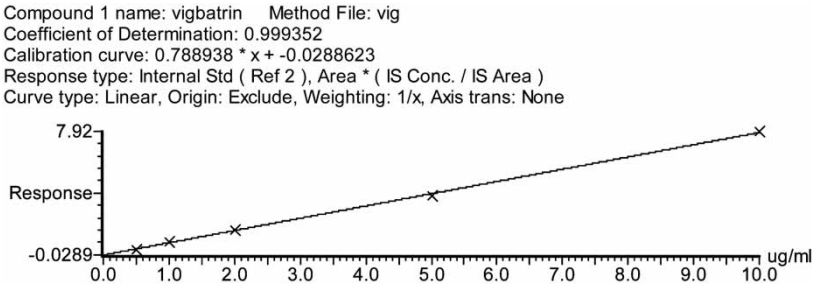


Figure 6. Calibration curve of VGB in human plasma after protein precipitation.

Table 2. Intra- and inter-day precision and accuracy for determination of VGB in human plasma by LC-MS/MS

Nominal concentration (μg/mL)	Found (mean ± S.D.) (μg/mL)	CV (%)	Dev. (%)[a]
Intra-day precision[b]			
0.75	0.71 ± 0.05	6.53	−4.93
3.0	2.94 ± 0.11	3.84	−2.03
7.5	7.34 ± 0.36	4.87	−2.15
Inter-day precision[c]			
0.75	0.80 ± 0.07	9.15	6.67
3.0	3.01 ± 0.08	2.78	0.40
7.5	7.13 ± 0.33	4.59	−4.91

[a]Dev. (%) = 100 × (Found concentration − Nominal concentration/Nominal concentration).
[b]Mean values represent eight different plasma samples for each concentration.
[c]Inter-day precision was determined from eight different runs over a 4 week period for each concentration.

Table 3. Recovery percentages of VGB from human plasma using LC-MS/MS

Nominal concentration (μg/mL)	Percentage recovery (Mean ± S.D., %)
0.75	99.17 ± 3.94
3.0	99.46 ± 3.88
7.5	100.15 ± 3.29

Table 4. Effect of freeze–thaw on VGB stability in human plasma

Nominal concentration (μg/mL)	(Mean[a] ± S.D.)	CV (%)
0.75	0.72 ± 0.07	9.14
3.0	2.94 ± 0.12	3.94
7.5	7.24 ± 0.28	3.90

[a]Mean values represent five cycles.

Table 5. Effect of frozen storage on VGB stability in human plasma at −20°C

Nominal concentration (μg/mL)	(Mean ± S.D., %)	CV (%)
0.75	94.67 ± 6.77	7.15
3.0	98.25 ± 2.36	2.40
7.5	96.23 ± 3.83	3.98

CONCLUSION

The present work reports on the utility of liquid chromatography for the analysis of VGB in human plasma using MS/MS detection. The high sample throughput, short-time of analysis, the absence of the need to have complete chromatographic separation, and simple sample preparation permit analysis of large number of samples of VGB including standards, QC, and analytes during pharmaceutical and clinical studies. The described LC-MS/MS method was validated and the results obtained showed good characteristics of specificity, sensitivity, and accuracy, which make it highly valuable in routine analysis of VGB in clinical practice, particularly in pharmacokinetics and bioavailability/bioequivalence studies.

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