Global Advanced Research Journal of Medicine and Medical Sciences (ISSN: 2315-5159) Vol. 5(2) pp. 042-052, February, 2016
Available online http://garj.org/garjmms

Full Length Research Paper

Optimized HILIC-MS/MS Method for Simultaneous Determination of two Antiepileptic Drugs with Significant Difference in Binding to Serum Albumin

Hassan A. Alhazmi[1*], Mohammed Al Bratty[1], Gunasekar Manoharan[1], Hafiz A. Makeen[2] and Adnan A. Kadi[3]

[1]Jazan University, College of pharmacy, Department of Pharmaceutical Chemistry, P.O. Box 114, Jazan 45142, Saudi Arabia.
[2]Jazan University, College of pharmacy, Department of Clinical Pharmacy, P.O. Box 114, Jazan 45142, Saudi Arabia.
[3]King Saud University, College of pharmacy, Department of Pharmaceutical Chemistry, P.O. Box 2457, Riyadh 11451, Saudi Arabia.

Accepted 04 February, 2016

Different HILIC columns were studied and compared. Each column showed different behaviors regarding to the separation of four antiepileptic drugs and the influence on MS/MS sensitivity through the ionization of the drugs. HILIC Luna was selected since it has been offered good separation and sensitivity. Additionally, a fast, sensitive and selective HILIC-MS/MS method was developed for the detection and quantification of two antiepileptic drugs, carbamazepine (highly bind to serum albumin) and vigabatrin (not significant bind to serum albumin) in human plasma. Samples were purified by two steps, precipitation by methanol and using the supernatant for vigabatrin and liquid–liquid extraction using ethylacetate for carbamazepine. The separation was successfully achieved on a HILIC LUNA 3µm (2× 150 mm) column with a mobile phase consisting acetonitril/ammonium formate (5mM, pH 3.5) (75:25, v/v). A triple quadrupole model G6410A mass spectrometerin the MRM mode was used for detection using electro spray ionisation (ESI). The detections were based on the transition of the protonated molecular ion for carbamazepine at *m*/*z* 237 and vigabatrin at *m*/*z* 130 to the predominant ions of *m*/*z* 194 and 71, respectively. The acceptable mean recoveries were 81% for carbamazepine and 100% for vigabatrin. Furthermore, the calibration curves were linear over the concentration range 250–8000 ng/mL for carbamazepine and 15–960 ng/ml for Vigabatrin. The LODs for carbamazepine and vigabatrin in human plasma were 5.43 ng/mL and 3 ng/mL, respectively.

Keywords: HILIC, MS/MS, Carbamazepine, Vigabatrin.

INTRODUCTION

Therapeutic drug monitoring (TDM) of antiepileptic drugs is always important to optimize the acceptable therapeutic level of the antiepileptic dugs in plasma including carbamazepine (CBZ) and vigabatrin (VGB) (Gross, 1998;Maresova et al., 2008). CBZ is an established drug for the control of grand mal and psychomotor epilepsy and is also effective in the

*Corresponding Author E-Mail: hasalhazmi@gmail.com; Phone: +966 541433344

treatment of trigeminal neuralgia. It has also been more recently used for the management of bipolar disorder (Van Rooyen et al., 2002). VGB is a structural analogue of γ-aminobutyricacid.VGB is used to treatment of resistant epilepsy, complex partial seizures, and secondary generalized seizures and for monotherapy use in infantile spasms in West syndrome (Kalviainen and Nousiainen, 2001; Chiron and Dulac, 2002; Curatolo et al., 2006).Several methods have been published for the determination of one or more antiepileptic drugs in biological fluids for TDM or for toxicological purposes. Several high-performance liquid chromatography (HPLC) methods with UV detection for determination of CBZ and its metabolites, including CBZ-E, in biological fluids and drug products are available in the literature (Van Rooyen et al., 2002; Zhu et al., 2005; Oh et al., 2006; Gupta et al., 2006;Moreno et al., 2004). LC MS MS has also been used for determination of CBZ and its metabolites (Van Rooyen et al., 2002; Subramanian et al., 2008). Various HPLC methods for the simultaneous determination of CBZ, phenytoin and Phenobarbital have been reported (Yoshida et al., 2006; Patil and Bodhankar, 2005; Liu et al., 1993; Romanyshyn et al., 1994; Kishore et al., 2003; Levert et al., 2002; Kouno et al., 1993). Simple chromatographic methods of UV detection for VGB are not applicable due to the lack of volatility and chromophore. Numerous HPLC methods have been published about the determination of VGB in biological fluids using different derivatizing reagents (Sagirli et al., 2006; Berry and Millington, 2005; Franco et al., 2007). In one case a simultaneous HPLC analysis of gabapentin, pregabalin and VGB in human serum by pre-column derivatization with o-phthaldialdehyd and fluorescence detection has been reported (Vermeij and Edelbroek, 2004). Methods with LC-MS/MS detection systems, where there is no need for derivatization have been reported for VGB (Matar and Abdel-Hamid, 2005; Kadi et al., 2013). However, there are no methods for quantification two antiepileptic drugs with significantly different in binding to serum albumins.

Hydrophilic interaction chromatography (HILIC) is a liquid chromatography (LC) technique that uses a polar stationary phase (for example, silica or a polar bonded phase) in conjunction with a mobile phase containing an appreciable quantity of water (usually at least 2.5% by volume) combined with a higher proportion of a less polar solvent (often acetonitrile) which lead to enhance sensitivity when used in conjunction with mass spectrometry (MS) because the high organic content of the mobile phase in HILIC allows for efficient spraying and desolvation in electrospray MS (Irgum, 2006). Most AED's are less polar compound and they can be eluted early which lead to decrease the run time of method.

MATERIALS AND METHODS

Materials and chemicals

Separation was performed on an Agilent 1200 series system consisting of G1311A Quaternary pump, G1332A Degasser, G1367B HIP-ALS Auto sampler, G1316B Thermostatted column compartment and G13115B DAD detector. Mass detection was performed on a model G6410A triple quadrupole (Agilent Technologies, USA). HILIC columns, HILIC Luna (3 µm, 2 mm I.D ×150 mm, Phenomenex, USA), Polar-100 (3 µm, 2.1 mm I.D ×150 mm, Sepax Tech., USA), Polar-imidazole (1.8 µm, 2.1 mm I.D ×50 mm, Sepax Tech., USA) and polar-Pyridine (1.8 µm, 2.1 mm I.D ×50 mm, Sepax Tech., USA). The mobile phase consisted of acetonitril/ ammonium formate (5mM, pH 3.5) (75:25, v/v) and was pumped at 0.4ml/min. Samples were introduced into a column at an injection volume of 5 µl. Acetonitril, methanol and diethylether were obtained from BDH Laboratory Supplies (poole, UK) and ACROS (USA), ethylacetate was obtained from Winlab laboratory supplies(UK). Analytical reagents grade ammonium formate, ammonium acetate, potassium carbonate, sodium acetate formic acid and acetic acid were obtained from BDH chemicals (Pool, UK). Water was purified by Millipore system (Milipore, France). The aqueous mobile phase was filtered through amilipore membrane (HA 0.45 µm). CBZ was supplied by Tabuk pharmaceutical industry (tabuk, Saudi Arabia). VGB, phenytoin (PHT) and lamotrigine (LTG) were purchased from Sigma Chemicals, (Saint Louis, MO). Glibenclamide was supplied by Spimaco (Al-qassim, Saudi Arabia). Human plasma has been obtained from King Khalid hospital (KSA, Riyadh).

Preparation of Calibration Standards

Primary stock solutions of CBZ and its IS (GLB) (100µg/ml) were separately prepared in acetonitril. The working standards of CBZ (2.25µg/ml, 4.5µg/ml, 9µg/ml, 18µg/ml, 36µg/ml and 72µg/ml) and IS (0.9µg/ml) were prepared by diluting each primary solution with mobile phase. The primary stock solution of VGB (100µg/ml) was prepared in water and the working standards (0.135µg/ml, 0.27µg/ml, 1.08µg/ml, 2.16µg/ml, 4.32µg/ml and 8.64µg/ml) were prepared by diluting the primary solution with mobile phase. No internal standard was used with VGB. Human plasma calibration standards and quality control standards containing CBZ and VGB were prepared by spiking appropriate amounts of working standard solutions in to drug-free human plasma and then serially diluting it with normal blank plasma to attain

the desired concentration range. The prepared calibration standards and quality control standards were prepared in glass ampoule's (5ml) and stored at -20 °C until analysis.

Sample Preparation

To 450 µl of plasma was added methanol (1ml) and IS (50µl, 150ng/ml in acetonitril) in a 5-ml glass ampoule (A). The samples were vortexed for 40 seconds and centrifuged at 5000 *g* for 5 min. The supernatant were transferred to another 5-ml glass ampoule (B). The precipitate protein was treated with 1ml of 0.1M sodium hydroxide and vortexed for 1min. Ethyl acetate (2 ml) was added and the samples were vortexed for 1min, the organic layer was aspirated and transferred to a 5-ml glass ampoule (B) and evaporated under a stream of nitrogen at 40°C. The residue was reconstituted in 450 µL of acetonitril: water (65:35), vortex-mixed for 50 seconds and 5 µL was introduced into the LC-MS/MS system for analysis.

Instrumental Conditions

Chromatography was performed at ambient temperature (25°C), at a flow-rate of 0.4 ml/min with acetonitrile/ammonium formate (5mM, pH 3.5) (75:25, v/v) as mobile phase. The aqueous chromatographic solvents were filtered through amilipore membrane (HA 0.45 µm) before use.

Mass Spectrometry

Electrospray ionisation was performed in the positive mode with the nebulizing gas (nitrogen), gas temperature**,** gas flow and capillary volt set at 50 psi, 350°C, 11l/min and 5500V, respectively. The instrument responses for CBZ and its internal standard GLB, and VGB were optimized using flow injection analysis. Optimal responses and transition of the protonated molecular ions are summarized in table 1.

Validation

Method validation was performed according to current international regulations on analytical method validation (ICH Guideline, 1996; FDA Bioanalytical Method, 2001).The method was validated by using plasma quality control samples (n=5) at 45, 480 and 768ng/ml for VGB and 750, 4000 and 6400 ng/ml for CBZ, to determine the accuracy and precision of the method. Quality control values were calculated from a standard regression curve, constructed from the ratio of analyte to internal standard peak areas for CBZ and peak area for VGB by using six different concentrations. The calibration curve was linear over the concentration range 250–8000 ng/mLfor CBZ and 15–960 ng/ml for VGB.

Matrix effects

Matrix effects were determined by analysing blank biological fluids from six different sources to determine possible interference.

Recovery

Absolute recovery of the analyte was determined in triplicates at high, medium and low concentrations in normal plasma by extracting drug free plasma samples spiked with CBZ and VGB. Recovery was calculated by comparison of the analyte peak-areas(peak ratio for CBZ) of the extracted samples with those of the unextracted analyte standards, representing 81 and 100% recovery respectively.

Stability

The stability of CBZ, GLB and VGB stock solutions were evaluated at room temperature for 6 hours, and after storage at –20°C for 40 days. All stock solutions were stressed by exposing them for ½ hr to UV light. Stability was calculated by comparing the pertinent responses obtained from the tested stock solution(s) with the responses of freshly prepared ones and the result are given in Table 4. Sufficient number of QC samples at each concentration level (45 and 768 ng/ml) for VGB and (750 and 6400 ng/ml) for CBZ were allocated to carry out the short-term stability. Six samples at each level were analyzed for initial concentration determination at zero time. Another QC samples at each level were left on the bench top at room temperature for 6 hours and then analyzed (6 QC's at each time interval). Moreover, QC samples at each level were exposed to UV light at room temperature for ½ hr and then analyzed. Stability was calculated by comparing the tested QC samples with those analyzed initially and the result are given in Table 4. To perform long-term stability, six QC samples at each concentration level (45 and 768 ng/ml) for VGB and (750 and 6400 ng/ml) for CBZ were stored at –70°C and –20°C for 30 days, then analyzed. Stability was evaluated by comparing stored samples with those analyzed initially and the result are given in table 4.

RESULTS AND DISCUSSION

Four HILIC columns with different stationary phases namely, HILIC Luna, Polar-100, Polar-imidazole and polar-Pyridine were studied and compared. LTG, CBZ, PHT and VGB were used as models. All parameters of LC-MS-MS were fixed during work since the evaluation was based on ions scan (extracted-ion chromatogram, EIC). The optimum mobile phase composition for

Table 1. MS of anticonvulsant drugs: used and found ions, MS conditions and used transitions

Substance	Molecular weight	Precursor ion	Fragmentor	Collision energy	Dwell time	Used transitions
CBZ	236.3	237	100	14	200	237 → 194
VGB	129.0	130	80	9	200	130.0 → 71 130.0 → 113
GLB	494.0	494.2	100	14	200	494.2 → 369.1 494.2 → 395

Table 2. Summary of intra-day quality control results for CBZ (*n*=5) and VGB (*n*=5)

(A) CBZ	**QH***	**QM**	**QL**
Nominal (ng/ml)	6400	4000	750
Mean	5924.41	3785.69	695.87
%Nom	92.57	94.64	92.78
RSD (%)	2.08	1.79	5.89
(B) VGB	**QH**	**QM**	**QL**
Nominal (ng/ml)	768	480	45
Mean	726.83	520.83	44
%Nom	94.64	108.50	97.78
RSD (%)	1.145	2.20	4.6

* QH, QM and QL are abbreviations of high, medium and low quality controls, respectively.

seperation was found to (be acetonitrile) /ammonium formate (5mM, pH 3.5) (75:25, v/v). The flow rate of 0.2ml/min was employed for this study. As shown in Figure 1, Polar-100 and HILIC Luna have similar stationary phase with one hydroxyl group bound to silica base stationary phase. This hydroxyl group offers weak acid stationary phase. The stationary phase of Polar-Diol is certainly more polar and acidic than Polar-100 since its stationary phase contains two hydroxyl groups bound to silica base stationary phase. Polar-Pyridine and Polar-Imidazole are basic phases. Furthermore, it is well known thatin general imidazole is more basic than pyridine. The results were collected and summarized in one overlapped chromatogram for each compound, LTG (Figure 2), CBZ (Figure 3), PHT (Figure 4) and VGB (Figure 5).

As shown in figures 2-5, all selected antiepileptic drugs were eluted fast within the same time of 1.25-1.5 min. This is due to the less polarity of pyridine HILIC column than other HILIC columns. LTG and VGB (more polar compounds) more retained in HILIC luna and Polar-100 compared to CBZ and PHT (less polar compound). This is due to the acidity and high polarity stationary phase of HILIC and Polar-100. Since polar-imidazole is more polar than polar-Pyridine, all antiepileptic drugs were more retained in polar-imidazole compared to polar-Pyridine. Furthermore, PHT is considered as acidic compound, and hence it was highly retained in polar-imidazole than CBZ.

In case of the sensitivity, the extracted-ion chromatogram (EIC) of each drug was changed when changing the HILIC column. In most cases, HILIC Luna has been offered good sensitivity since it was enhanced the ionization of drugs. Hence, investigate the influence of different HILIC columns on MS-MS sensitivity can be fast investigated when required. In this study, HILIC Luna was selected to develop a new method for determining two compounds (CBZ and VGB) with different polarity.

The method was accelerated by increasing the flow rate of the mobile phase to 0.4ml/min. Therefore, the observed typical retention times for CBZ and its internal standard were 1.2–1.3 min (mean RSD 0.658%) and 1.1-1.2 (mean RSD 7.4%), and for VGB were 2.1–2.2 min (mean RSD 3.79%). In general, a total run time less than 3 min made it possible to analyse more samples per day. The plasma containing CBZ and VGB were extracted by using different pH modifier such as potassium carbonate and sodium acetate. The extracts were found to be dirty with low peak area of analytes. The extraction with ethylacetate for CBZ and its IS gave the best results and it was decided to optimize this extraction. The procedure for good extraction of less polar compound such as CBZ and highly polar compound such as VGB from plasma is

Table 3. Summary of back-calculated quality control concentrations of CBZ and VGB (inter-day variation) showing the repeatability of the method

(A) CBZ	**QH**	**QM**	**QL**
Nominal (ng/ml)	6400	4000	750
Mean	5958.98	3918.9	674.64
Accuracy (%)	93.10	97.97	89.95
RSD (%)	2.98	4.77	5.626
(B) VGB			
	QH	**QM**	**QL**
Nominal (ng/ml)	768	480	45
Mean	710.9	516.1969	44.18
Accuracy (%)	92.57	107.54	98.19
RSD (%)	3.761	7.03	4.28

Table 4. Summary of stability of CBZ, GLB and VGB in stock solution and human plasma.

Data of Stock Solution Stability.			
Drug(n=5)	**6 hrs at RT**	**0.50 hr under UV**	**40 Days at –20°C**
CBZ			
Precision (%)	0.60	2.76	2.10
Accuracy (%)	100.53	99.08	99.62
GLB			
Precision (%)	1.33	1.11	1.04
Accuracy (%)	97.89	97.87	97.07
VGB			
Precision (%)	1.73	1.63	0.45
Accuracy (%)	100.65	102.07	103.68
Data of Stability in Plasma Samples at Low and High Levels.			
Drug(n=5)	**6 hrs at RT**	**0.50 hr under UV**	**25 Days at –20°C**
CBZ			
750ng/ml			
Precision (%)	1.45	1	0.48
Accuracy (%)	98.50	99.25	79.72
6400ng/ml			
Precision (%)	0.18	1.18	0.91
Accuracy (%)	102.17	100.31	77.79
VGB			
45ng/ml			
Precision (%)	1.03	0.73	1.52
Accuracy (%)	96.94	99.62	78
768ng/ml			
Precision (%)	0.69	0.48	1.85
Accuracy (%)	96.25	96.99	76.66
GLB			
16.6ng/ml			
Precision (%)	0.84	0.55	2.98
Accuracy (%)	101.75	103.58	80

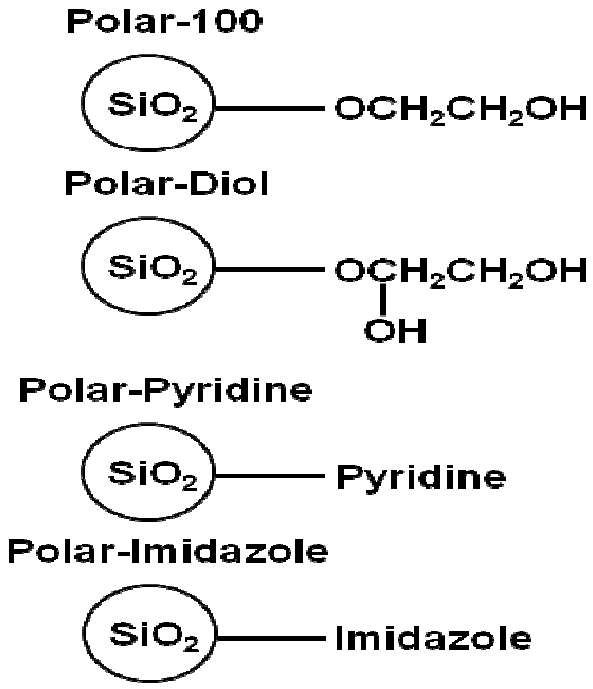

Figure 1. Chemical structure of stationary phases of different commercially available HILIC columns.

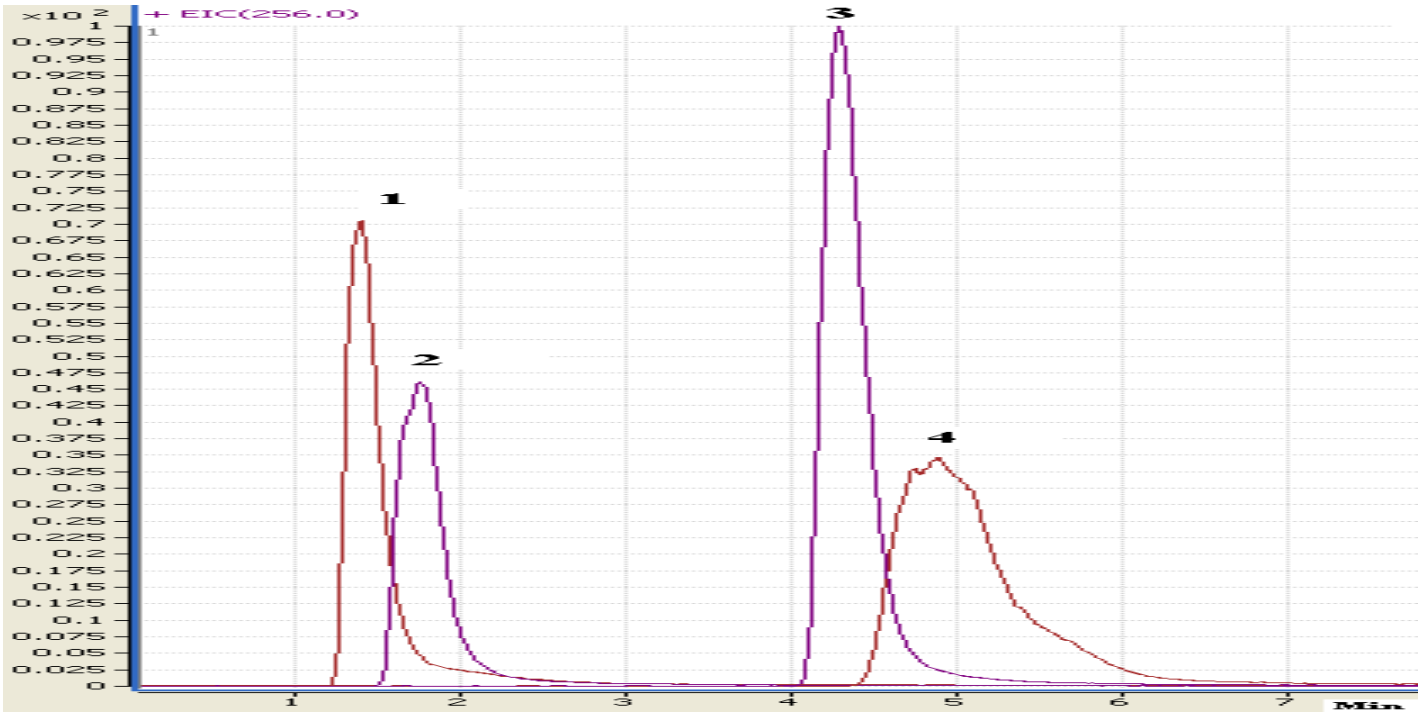

Figure 2. Peaks of 1, 2, 3 and 4 show elution of LTG using polar-Pyridine, polar-imidazole, HILIC Luna and polar-100, respectively.

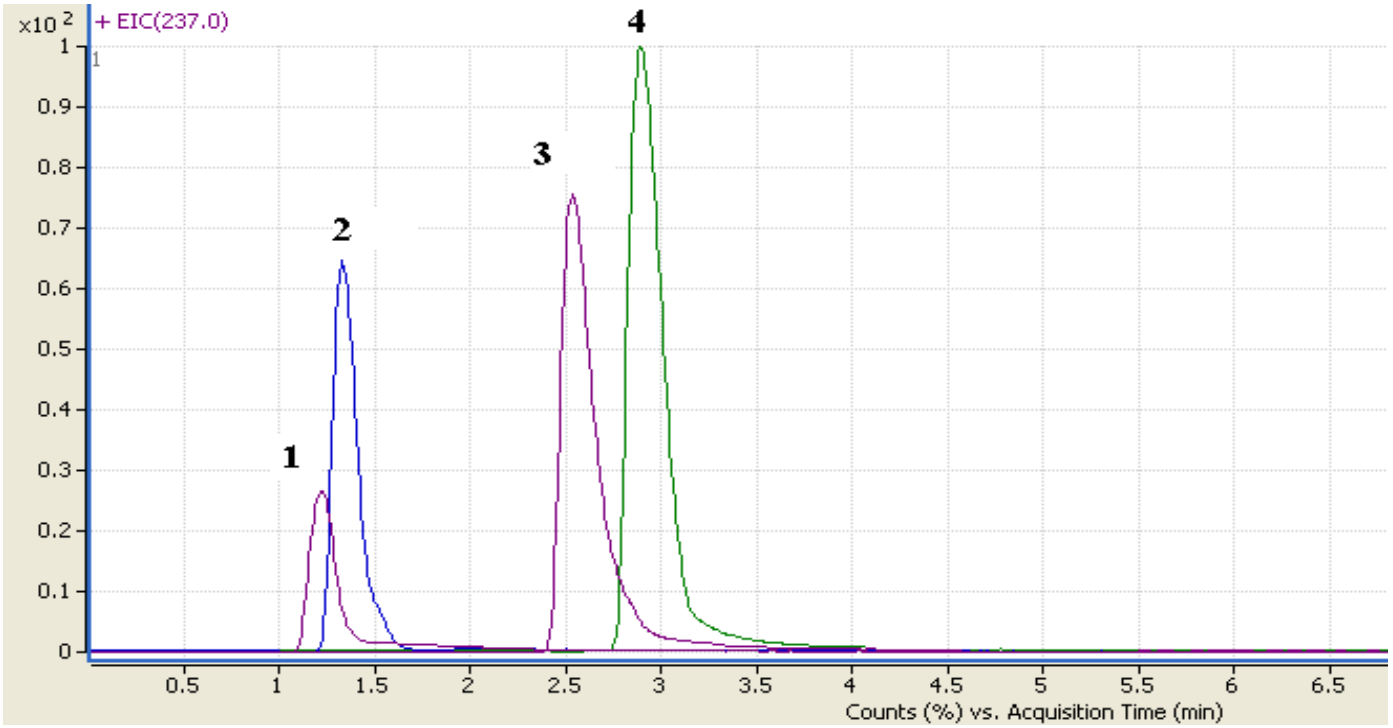

Figure 3. Peaks of 1, 2, 3 and 4 show elution of CBZ using polar-Pyridine, polar-imidazole, polar-100 and HILIC Luna, respectively.

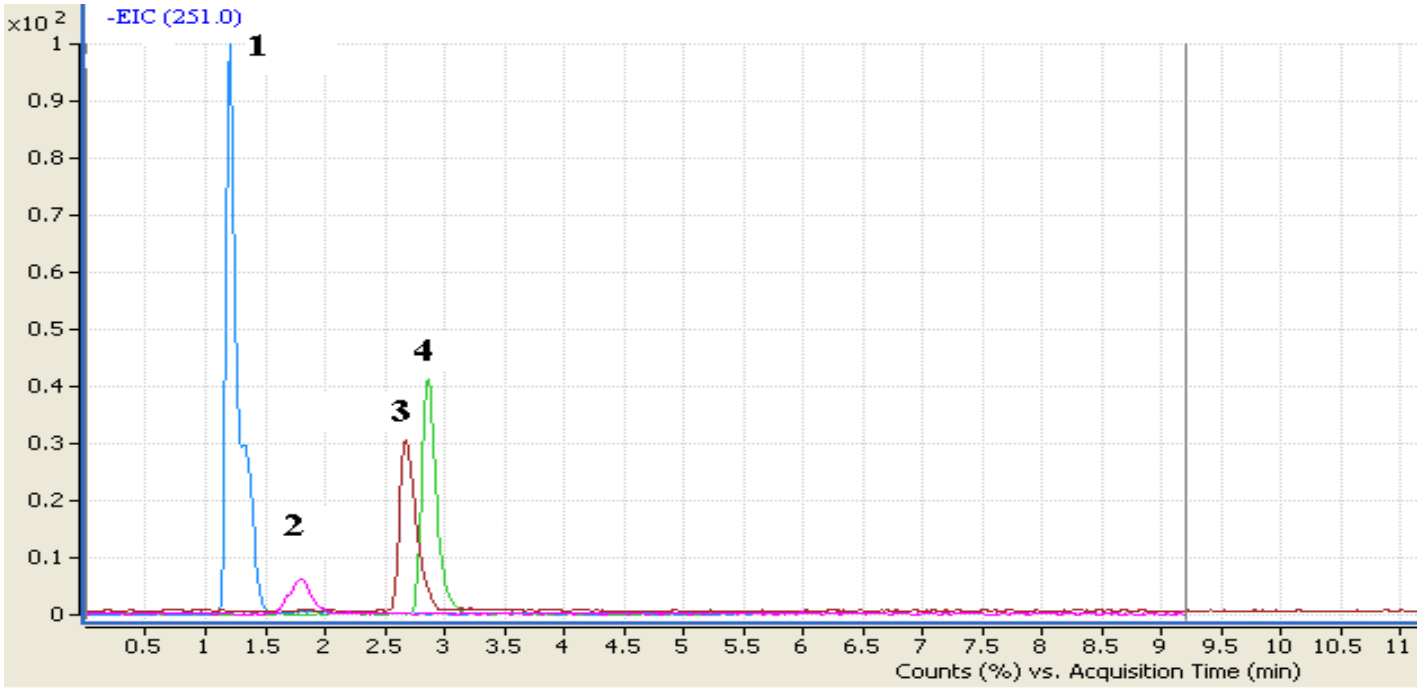

Figure 4. Peaks of 1, 2, 3 and 4 show elution of PHT using polar-Pyridine, polar-imidazole, polar-100 and HILIC Luna, respectively.

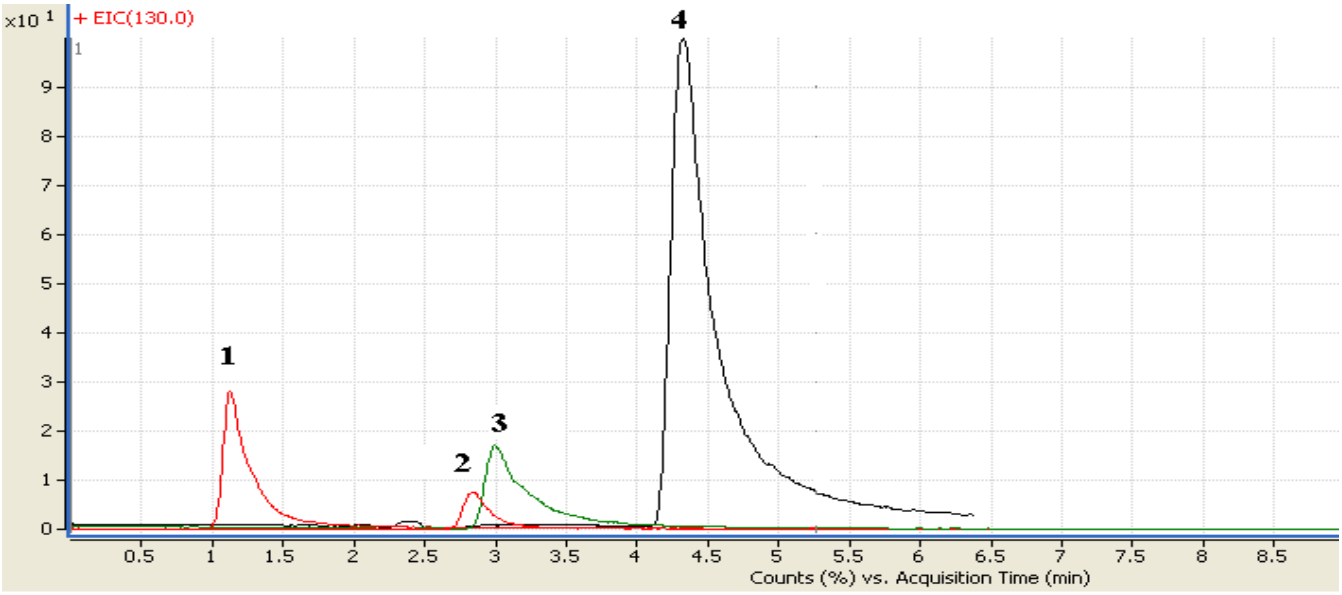

Figure 5. Peaks of 1, 2, 3 and 4 show elution of VGB using polar-Pyridine, polar-imidazole, polar-100 and HILIC Luna, respectively.

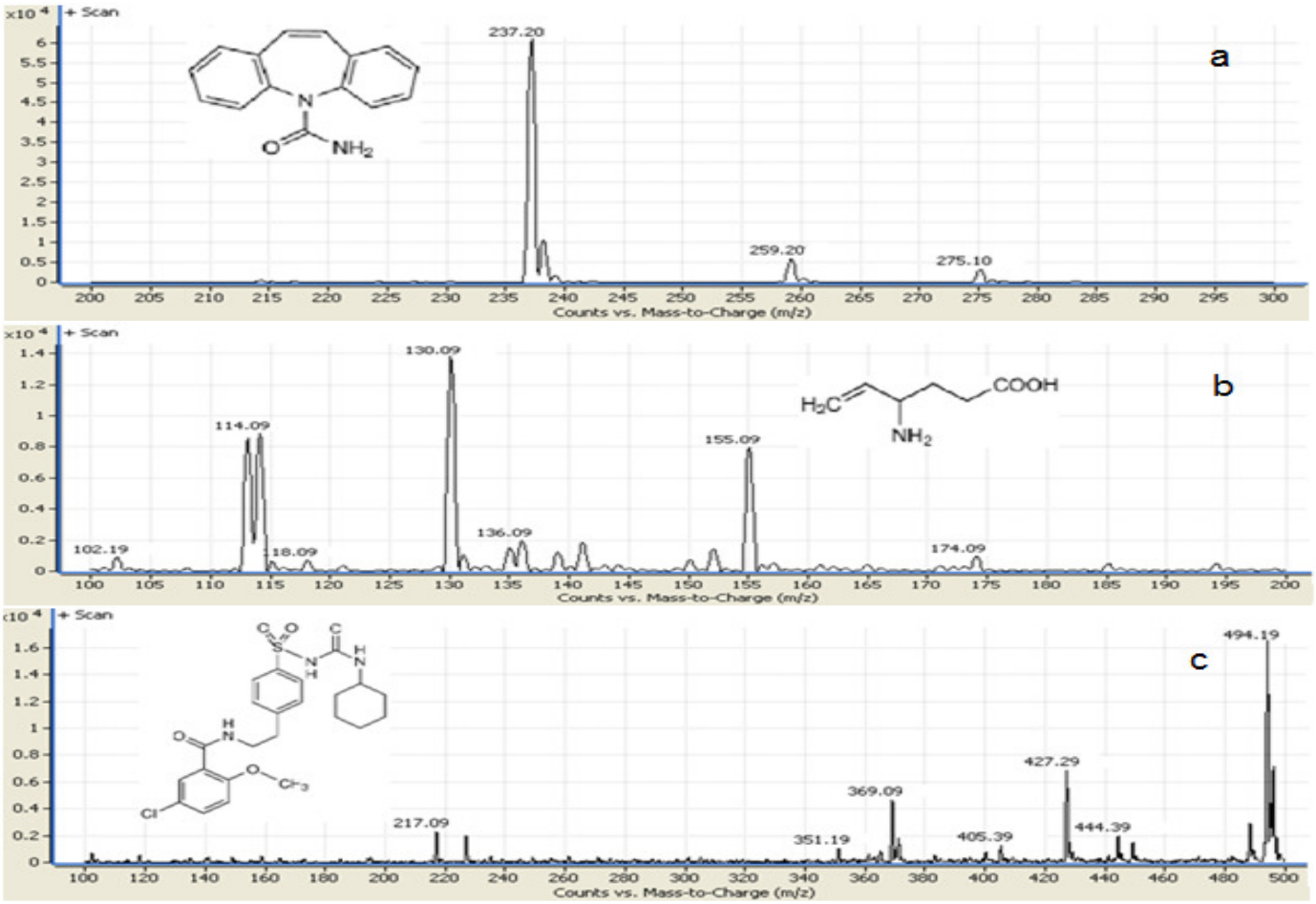

Figure 6. (a) A MS2 Scan spectrum of a pure solution of CBZ in acetonitrile/ammonium formate (5mM, pH 3.5) (75:25, v/v). Parent [M+1] ion with *m/z* 237.20 is shown. (b) A MS2 Scan spectrum of a pure solution of VGB in acetonitrile/ammonium formate (5mM, pH 3.5) (75:25, v/v). Parent [M+1] ion with *m/z* 130.09 is shown. (c) A MS2 Scan spectrum of a pure solution of GLB in acetonitrile/ammonium formate (5mM, pH 3.5) (75:25, v/v). Parent [M+1] ion with *m/z* 494.19 is shown.

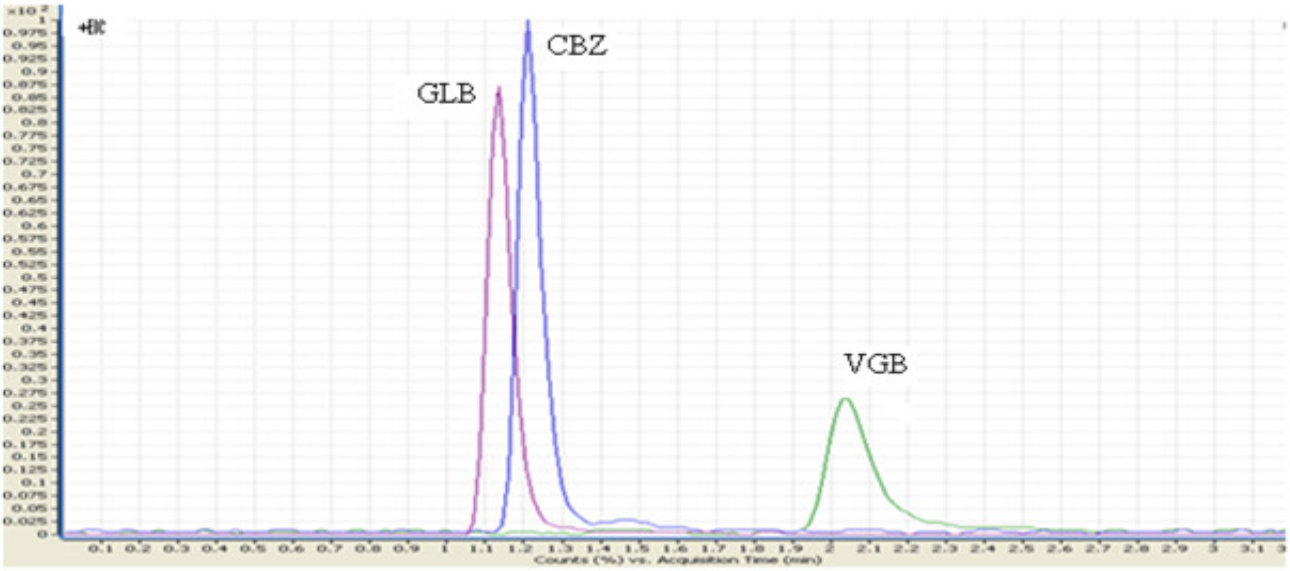

Figure 7. Extracted ion chromatogram (EIC) of GLB, CBZ and VGB

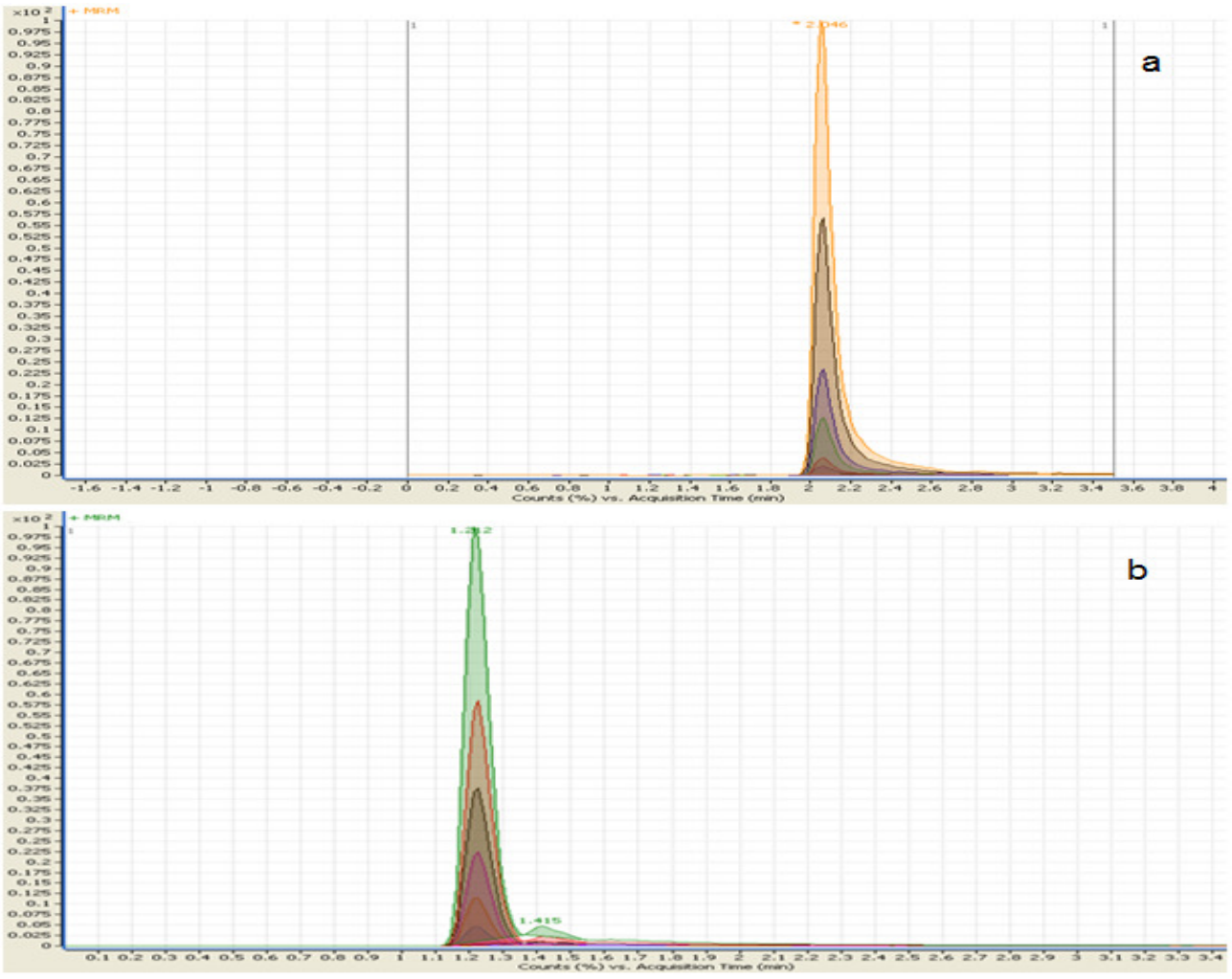

Figure 8. (a) Peaks of six different concentrations of vigabatrin were used for plasma calibration curve. (b) Peaks of six different concentrations of carbamazepine were used for plasma calibration curve.

discussed in details in section 2.3. Figure 6a, b and c shows a full scan spectrum (MS2Scan) spectra for CBZ, VGB and CLB with *m*/*z* values of 237.20, 130.09 and 494.19 representing protonated molecular ions peaks of the three analytes, respectively.

Figure 7 shows representative multiple reactions monitoring (MRM) chromatogram of CBZ, GLB and VGB. The GLB was used to calculate peak ratios of VGB and the results weren't linear. For this reason, the analysis of VGB was successfully done without IS. The calibration curve was linear (r=0.991) over the concentration range 250–8000 ng/mL for CBZ and 15-960 ng/ml (r=0.999) for VGB.

Figure 8(a) shows representative MRM chromatograms of CBZ corresponding to the six different calibration standard concentrations (250, 500,1000, 2000, 4000 and 8000ng/ml). Figure 8(b) shows representative MRM chromatograms of VGB corresponding to the six different calibration standard concentrations (15, 30,120, 240, 480 and 960ng/ml). Specificity was evidenced by the lack of interfering peaks in the chromatograms of plasma samples. Figure 9 shows a MRM chromatogram for a blank plasma sample indicating the methods' specificity.

Table 2 shows the quality controls data obtained during the validation of the method for CBZ and VGB, while Table 3 shows the intra-day back calculated quality controls for CBZ and VGB. Both intra-and inter-assay CV values ranged from 1.79–5.89% at three QC levels (i.e., 750, 4000 and 6400 ng/ml.) for CBZ and 1.145-7.03% at three QC levels (i.e., 45, 480 and 768ng/ml.) for VGB.These results indicate that the present method has acceptable accuracy and precision. The LODs for CBZ and VGB in human plasma were 5.43 ng/mL and 3 ng/mL, respectively. The LOQs for both analytes in human plasma were 18.2 ng/ml and 10 ng/ml, respectively using 5µL of human plasma. Data of Stock Solution Stability and Data of Stability in Plasma Samples at Low and High Levels for VGB, CBZ and IS are presented in table 4.

CONCLUSION

Different HILIC columns were studied. Each column showed different behaviors regarding to the separation of four antiepileptic drugs and the influence on MS-MS sensitivity through the ionization of a drug. HILIC Luna was selected to develop a new method for determination of two antiepileptic drugs with different polarity. Therefore, a highly sensitive, fast and selective method for the detection and quantification of CBZ and VGB in human plasma has been developed and validated by using HILIC Luna coupled to triple quadrupole mass spectrometer. To the best of the authors' knowledge, this method is the first reported for simultaneous quantitation of two widely prescribed antiepileptic agents, with one highly protein binding drug (CBZ) and the other (VGB) with significantly less degree of protein binding. A chromatography time of 3 min made it possible to analyze more samples per day.

ACKNOWLEDGEMENT

We gratefully acknowledge King Abdul Aziz City for Science and technology for providing the financial support and required facilities to carry out this research work through a scientific research grant.

REFERENCES

Berry D, Millington C (2005). Analysis of pregabalin at therapeutic concentrations in human plasma/serum by reversed-phase HPLC. Ther. Drug Monit. 27: 451–456,

Chiron C, Dulac O (2002). Drug therapy for West's syndrome. Adv. Exp. Med. Biol. 497:51–56.

Curatolo P, Bombardieri R, Cerminara C (2006). Current management for epilepsy in tuberous sclerosis complex. Curr. Opin. Neurol. 19:119–123.

FDA Bioanalytical Method Validation Guidelines (2001). http://www.fda.gov/ cder/guidance/index.html.

Franco V, Mazzucchelli I, Fattore C, Marchiselli R, Gatti G, Perucca E (2007). Stereoselective determination of vigabatrin enantiomers in human plasma by high performance liquid chromatography using UV detection. J. Chromatogr. B. 854: 63 – 67.

Gross AS (1998). Best practice in therapeutic drug monitoring. Br. J. Clin. Pharmacol. 46:95–99

Gupta M, Kohli K, Kumar D, Gupta YK (2006). A reverse phase high performance liquid chromatography method for simultaneous estimation of melatonin, carbamazepine epoxide and carbamazepine simultaneously in serum. Indian J. Phys. Pharmacol. 50(4): 427-430.

ICH Guideline (1996). Guidelines for Validation of Analytical Procedures Methodology. http://www.pharmweb.net/pwmirror/pw9/ifpma/ich1.html (step 4).

Irgum P (2006). Review of hydropilic interaction chromatography. J. Sep. Sci. 29:1784-1821.

Kadi AA, Kassem MG, Makeen HA, Alhazmi HA (2013). Simultaneous determination of phenytoin and lamotrigine in human plasma using hydrophilic interaction liquid chromatography-triple quadrupole mass spectrometry. Digest J. Nanomaterials and Biostructures. 8(3): 1113 – 1122.

Kalviainen R, Nousiainen I (2001). Visual field defects with vigabatrin: epidemiology and therapeutic implications. CNS Drugs.15:217–230.

Kishore P, Rajnarayana K, Reddy MS, Vidyasagar J, Krishna DR (2003). Validated high performance liquid chromatographic method for simultaneous determination of phenytoin, phenobarbital and carbamazepine in human serum. Arzneimittel- forschung. 53:763-768.

Kouno Y, Ishikura C, Homma M, Oka K (1993). Simple and accurate high-performance liquid chromatographic method for the measurement of three antiepileptics in therapeutic drug monitoring. J. Chromatogr. 622:47-52.

Levert H, Odou P, Robert H (2002). Simultaneous determination of four antiepileptic drugs in serum by high-performance liquid chromatography. Biomed. Chromatogr. 16:19-24.

Liu H, Delgado M, Forman LJ, Eggers CM, Montoya JL (1993). Simultaneous determination of carbamazepine, phenytoin, phenobarbital, primidone and their principal metabolites by high-performance liquid chromatography with photodiode-array detection. J. Chromatogr. 616:105–115.

Maresova V, Chadt J, Novakova E (2008). Screening and semiquantitative analysis of drugs and drugs of abuse in human serum samples using gas chromatography-mass spectrometry. Neuro. Endocrinol. Lett. 29:749–754.

Matar KM, Abdel-Hamid ME (2005). Quantification of Vigabatrin in Human Plasma by Liquid Chromatography–Electrospray Tandem Mass Spectrometry. J. Liq. Chromatogr. Relat. Technol. 28:395 – 406.

Moreno J, Belmont A, Jaimes O, Santos JA, Lopez G, Campos MG, Amancio O, Perez P, Heinze G (2004). Pharmacokinetic study of carbamazepine and its carbamazepine 10,11-epoxide metabolite in a group of female epileptic patients under chronic treatment. Arch. Med. Res. 35:168-171.

Oh EK, Ban E, Woo JS, Kim CK (2006). Analysis of carbamazepine and its active metabolite, carbamazepine-10, 11-epoxide, in human plasma using high-performance liquid chromatography. Anal Bioanal Chem. 386:1931–1936.

Patil KM, Bodhankar SL (2005). Simultaneous determination of lamotrigine, phenobarbitone, carbamazepine and phenytoin in human serum by high- performance liquid chromatography. J. Pharm. Biomed. Anal. 39:181-186.

Romanyshyn LA, Wichmann JK, Kucharczyk N, Shumaker RC, Ward D, Sofia RD (1994). Simultaneous determination of felbamate, primidone, Phenobarbital, carbamazepine, two carbamazepine metabolites, phenytoin and one phenytoin metabolite in human plasma by high-performance liquid chromatography. Ther. Drug Monit. 16:90–99.

Sagirli O, Cetin SM, Onal A (2006). Determination of gabapentin in human plasma and urine by high-performance liquid chromatography with UV-VIS detection. J. Pharm. Biomed. Anal. 42: 618-624.

Subramanian M, Birnbaum AK, Remmel RP (2008). High-speed simultaneous determination of nine antiepileptic drugs using liquid chromatography-mass spectrometry. Ther. Drug Monit. 30:347–356.

Van Rooyen GF, Badenhorst D, Swart KJ, Hundt HK, Scanes T, Hundt AF (2002). Determination of carbamazepine and carbamazepine-10, 11-epoxide in human plasma by tandem liquid chromatography–mass spectrometry with electrospray ionisation. J. Chromatogr.B. Anal. Technol. Biomed. Life Sci. 769(1): 1–7.

Vermeij TA, Edelbroek PM (2004). Simultaneous high-performance liquid chromatographic analysis of pregabalin, gabapentin and vigabatrin in human serum by precolumnderivatization with *o*-phtaldialdehyde and fluorescence detection. J. Chromatogr. B. 810 (2): 297–303.

Vermeij TAC, Edelbroek PM (2004). Simultaneous High Performance Liquid Chromatographic Analysis of Pregabalin, Gabapentin and Vigabatrin in Human Serum by PrecolumnDerivatization with O-phthaldialdehyde and Fluorescenc Detection. J. Chromatography B: Biomedical Sciences and Applications. 810:297-303.

Yoshida T, Imai K, Motohashi S, Hamano S, Sato M (2006). Simultaneous determination of zonisamide, carbamazepine, carbamazepine-10, 11-epoxide in infant serum by high-performance liquid chromatography. J. Pharm. Biomed. Anal. 41:1386–1390 K.

Zhu Y, Chiang H, Wulster-Radcliffe M, Hilt R, Wong P, Kissinger CB, Kissinger PT (2005). Liquid chromatography/tandem mass spectrometry for the determination of carbamazepine and its main metabolite in rat plasma utilizing an automated blood sampling system. J. Pharm. Biomed. Anal. 38(1): 119–125.