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UFLC Method Development for the Quantification of Carbamazepine in Blood Samples and an Application to Pharmacokinetic Study

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ABSTRACT: An isocratic simple rapid method has been developed and validated for the determination of carbamazepine (CBZ) in both solution form and blood samples. The assay was performed using an analytical C18 (100 mm×4.6 mm i. d) with a mobile phase consisting of methanol and water (50:50), the flow rate was 1 ml/min and UV detection at 285 nm. The method was found to be specific for CBZ, no interfering peaks were observed with an overall analytical run time of 5 min. Accuracy reported as % recovery were found to be 95.83–103.34% and 93.18–104.49% for inter-day and intra-day accuracies, respectively. Inter-day precision (reproducibility) was found to be 0.22–1.19 SD, while intra-day precision (repeatability) was found to be 0.14–2.12 SD for the samples studied. The calibration curve was found to be linear with the equation y=13474x+16969, with a correlation coefficient of 0.997 (R2) over a concentration range of 0.5–40 lg/ml. The farthest point of quantitation was the least fixation. The system is straightforward and fast and does not require any preparatory treatment of the specimen. The strategy was approved and effectively requested the blood tests to study the medication pharmacokinetic parameters.

KEYWORDS: Carbamazepine; Validation; Blood samples; HPLC method

I. INTRODUCTION

Carbamazepine (CBZ), 5-H-dibenze [b, f] azepine-5-carboxamide (Fig. 1), is a tricyclic lipophilic exacerbate that is a first medication of decision for the treatment of basic and complex incomplete seizures of epilepsy (Duche and Loiseau, 1995). It is totally metabolized in the body and its remedial focuses have been accounted for as 6–12 µg/ml, albeit significant varieties may emerge (Goodman et al., 2001). The utilization of CBZ in epilepsy treatment constrained by its shifting fixations in blood, the vicinity of carbamazepine-10, 11-epoxide (CBZ-E), its dynamic metabolite that essentially adds to the adequacy, harmfulness and pharmacological movement as an anticonvulsant (He et al., 1992), however it achieves lower focuses than CBZ. . Neumorous techniques have been accounted for the determination of CBZ, utilizing chromatography (Ashy et al., 1986; Hartley et al., 1986; Chelberg et al., 1988; Bhatti et al., 1998; Dasgupta et al., 1999; Raggi et al., 2000; Franceschi and Furlanut, 2005; Oh et al., 2006; Yoshida et al., 2006; Elizabeth et al., 2007; Budakova et al., 2008; Hemenway et al., 2010). Elite fluid chromatography (HPLC) and fluorescence polarization immunoassay (FPIA) are typically utilized as normal strategies for the determination of this and different anticonvulsants (Sanchez et al., 1999). Different methods, for example, micellar electrokinetic fine chromatography (MECC) (Lancas et al., 2003) and chemiluminescence (Lee et al., 2003) were utilized. As of late, two methodologies were exhibited in the writing. One of them is spectrophotometry and multivariate alignment (Rezaei et al., 2005) for the synchronous determination of CBZ and phenytoin. The other strategy misuses the unordinary fluorescence of CBZ on a nylon layer (Escandar et al., 2004). Synchronous determinations of CBZ and its metabolites in natural liquids and medication items (Burke and Thenot, 1985; Owen et al., 2001) have been distributed, including, solid–liquid extraction (Wad, 1984), liquid–liquid extraction (Rouan et al., 1992), section exchanging (Juergens, 1984), deproteinization (Liu et al., 1993), spectrofluorimetry strategy (Huang et al., 2002), gas–liquid chromatography (Chen and Bashi, 1991), FT-Raman spectroscopy (Auer et al., 2003), planar chromatography (Mennickent et al., 2009),

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blendbar-sorptive extraction and superior fluid chromatography-UV discovery (SBSE/HPLC-UV) (Queiroz et al., 2008) and elite slim layer chromatography (HPTLC) (Patel et al., 2011). Likewise, LC–mass spectrometry strategies (Breton et al., 2005; Van Rooyen et al., 2002; Miao and Metcalfe, 2003; Zhu et al., 2005) have been accounted for the discovery of CBZ and its metabolites in amphibian situations and in plasma.

Along these lines, the point of this study was to build a strategy taking into account UPLC-UV that is fit for dissecting CBZ in both arrangement and blood tests keeping in mind the end goal to encourage transport investigations of CBZ.

II. MATERIALS AND METHODS

2.1. Materials

Carbamazepine was kindhearted supplied by Dr Reddy's examination offices, Hyderabad, Andhra pradesh. Methanol (HPLC assessment) was gained from E. Merck private confined, Mumbai. Water for HPLC was masterminded in examination focus. Each and every other substance and solvents were of investigative reagent grade.

2.2. Equipment and chromatographic conditions

The study employed aultra performance liquid chromatography (Schimadzu isocratic controller, Koyoto, Japan) equipped with a LC20AT Absorbance PDA detector and an 20µl capacity loop containing sampling system. The mobile phase consisted of methanol and water (50:50), and the flow rate was 1 ml/min. Separation was achieved using a 100 mm • 4.6 mm (i.d.) C18, Analytical, reversed phase column with an average particle size of 10 lm, and the column was kept at ambient temperature. The column effluent was monitored at 285 nm and the chromatographic dataanalysis was performed with the LC Solutions software (Schimadzu, Koyoto, Japan)

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2.3. Stocks solutions and standards

Stock arrangements of CBZ were arranged in triplicate by dissolving 50.0 mg CBZ in 50 ml methanol, bringing about an answer containing 1 mg/ml. This arrangement was weakened 20-fold by methanol to give working arrangement (50µg/ml). Working arrangements of CBZ (50µg/ml), were arranged by weakening of the stock arrangements in methanol. Adjustment bends were built in methanol by setting up a progression of centralizations of the medication (3.125, 6.25 12.5, 25, and 50 µg/ml). Adjustment bend was additionally developed in blood tests. These included recreate investigation of tests spiked with shifting centralizations of CBZ (3.125, 6.25 12.5, 25, and 50µg

2.4. Calibration curve in serum

Primary stock solution of 1 mg/mL of carbamazepine and phenobarbitone were prepared in methanol and stored at 4o C. Appropriate dilutions of carbamazepine were made in methanol to produce working stock solutions of 100, 10, and 1µg/mL. Different concentrations (1, 5, 10, 50, 100, 500 and 2500 µg/mL) of carbamazepine in serum were prepared for calibration curve. The samples were treated as stated in extraction procedure. The peak height ratios ob tained at different concentrations of the drug and IS were plotted against the concentration of drug. The slope of the plot determined by the method of least square regression analysis was used to calculate the carbamazepine concentration in the unknown sample. The calibration curve in the range of 1-100 µg/mL resulted in the regression equation y = 0.3799x+0.0327 (R2=0.99) in serum. The calibration curve of carbamazepine in rat serum is shown in Fig.2.
Tables 1 and 2 present the percentage of drug recovered relative to the nominal values.

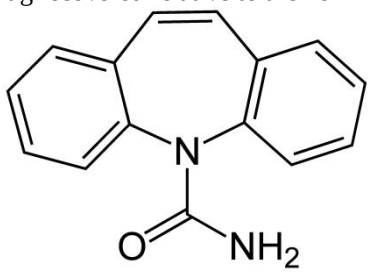

Figure 1: Chemical structure of carbamazepine

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2.4. Treatment of blood samples

The working arrangements (100 µl) were independently exchanged to clean dry centrifugation tubes. Blood test (500 µl) was added to every tube. These examples were vortex blended before centrifugation at 5000 rpm for 5 min. serum was differentiated. These were vortexed and blended for 2 min prior and then afterward including 400 µl of methanol. These specimens get hastened and this precipitation differentiated by centrifugation at 7000rpm for 10min. The divided supernatant was gone through layer channel 0.22µm and it stacked into HPLC vials before infusing 50 µl into the HPLC frame

2.5. Method validation

The adjustment bend of CBZ is a plot of the top range of the medication and the medication focus (C). This gives the accompanying mathematical statement: Peak range = Slope • C+ Intercept. The incline and the capture are resolved from the decided crest range and the ostensible convergance of the medication. The obscure CBZ focuses are dead set from this mathematical statement. The exactness of the technique in view of intraday variability was controlled by repeat examination of the adjustment models around the same time. The reproducibility was taken as the between day variability and was dictated by repeat examination of the adjustment principles in distinctive days with one imitate being examined every day. The standard deviation (SD) to the mean was calculated and recovery expressed as percentage. The accuracy of the method was determined by comparing practical amounts recovered from the control samples with actual values present in the samples (theoretical values). The selectivity of the method was determined by examining the interference from the endogenous materials in blood samples or from the degradation products of the drug. The farthest point of evaluation LOQ was taken as the most reduced fixation that can be precisely (relative mistake <2 for natural examples and <2 for the in vitro tests) and decisively decided (SD <2 for organic specimens and <2 for the in vitro tests). The LOD for CBZ was discovered to be 0.25µg/ml and this outcome is in concurrence with information reported in Elizabeth et al. (2007). The medication is stable in fluid arrangement and the examine was not accepted as dependability showed (Srinivasa and Belorkar, 2010)

2.6. In vivo bioavailability study in male wistar rats

The creature study convention was surveyed and endorsed by the institutional creature moral committee,Vaagdevi school of drug store, Kakatiya University, India. Male wistar rats measuring 200 to 225 g were chosen for study. The bioavailability investigation of carbamazepine was directed with an oral arrangement (carbamazepine 10 mg Kg -1 in 0.25%w/v CMC suspension). They were permitted free access to sustenance and water, until night before dosing and were fasted for 10 h. Oral arrangement (2.5 mg mL-1) was directed through sustaining needle. Blood tests (1.5 mL) from tail vein were gathered at preset interims of 0.0, 0.5, 1, 2, 3, 6, 12 and 24 h individually. All blood tests were permitted to cluster and centrifuged for 10 min at 4000 rpm. The serum was divided and moved into clean iniaturized scale axis tubes and put away at -20° C until HPLC examination. The measure of carbamazepine in the specimens was evaluated utilizing HPLC

III. RESULTS AND DISCUSSION

3.1. UFLC assay of CBZ

UFLC with UV detection was chosen as a simple, fast, and effective separation method for the determination of CBZ. In extensive preliminary experiments, a series of aqueous mobile phases with different pH values in combination with different organic modifiers were tested. Best results were obtained when using methanol and water (50:50) and adjusting the pH of the solution to 5, allowing adequate separation of the drug and the internal standard using a C18 Analytical column at a flow-rate of 1.0 ml/min. In addition, depending on the weak tissue protein binding of drug, methanol was used for protein precipitation (1:1) in order to obtain satisfactory values for recovery of CBZ. The selected chromatographic conditions provided optimum resolution of CBZ comparision to previous methods (28-39).

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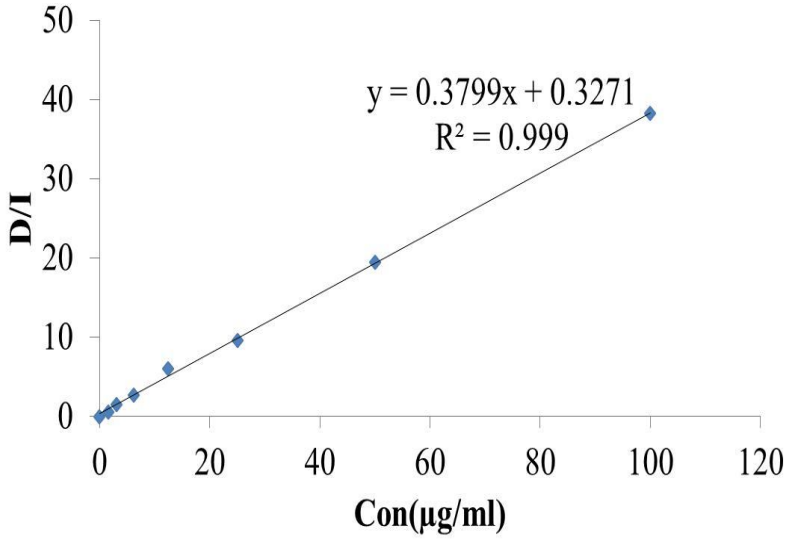

Figure 2: Calibration curve of carbamazepine in serum

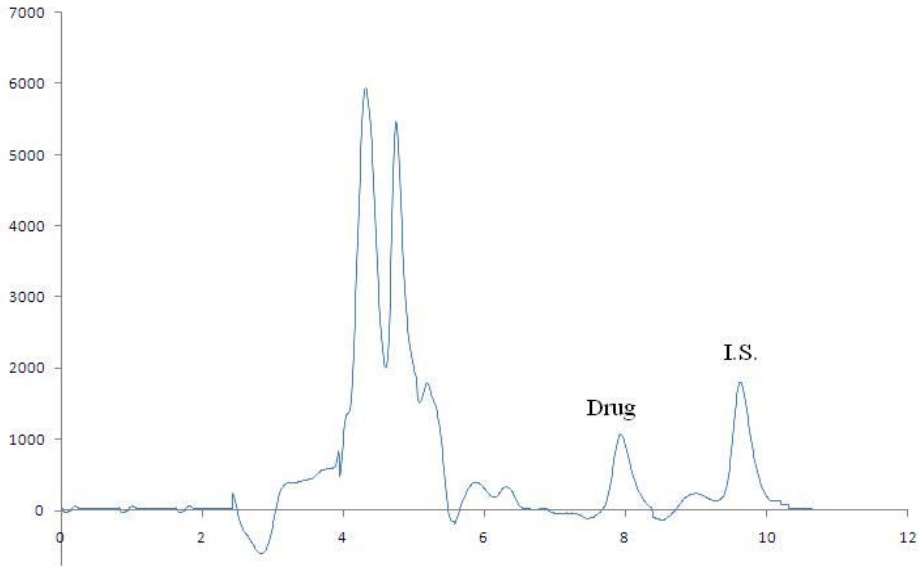

Figure 3: Chromatogram of carbamazepine in serum

Using the chromatographic conditions described above, the selectivity was further evidenced by the ability of the assay to separate the drug and the internal standard from plasma samples without interference from any endogenous material with a good separation and resolution of their peaks. This is clearly indicated from Fig. 3. The retention time of drug determined as 8.01minutes and internal standard retention time determined as 9.8 minutes.

In rat's bioavailability study, control group mean ± s.d. pharmacokinetic parameters like Cmax, T1/2, Auc0-∞, Vd, Cl, AUMC and MRT were determined as 57.71 ± 6.12µg/ml, 12.89 ± 1.25hr, 787.82 ± 90.72 µg/ml/hr, 34.69 ± 3.48ml, 0.033 ± 0.0012 ml/min, 19747.63 ± 274.67 µg/ml/hr2 and 25.06 ±1.41hr respectively.

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Time(hr)	Con(µg/ml)
0	0
0.5	21.35±2.7
1	31.34±3.5
2	42.04±5.6
3	57.71±4.8
4	46.29±3.4
6	32.02±4.2
8	26.76±3.1
10	16.99±2.7
12	12.74±2.1
24	9.73±1.5

Table 1: Serum concentrations of Carbamazepine in µg/ml

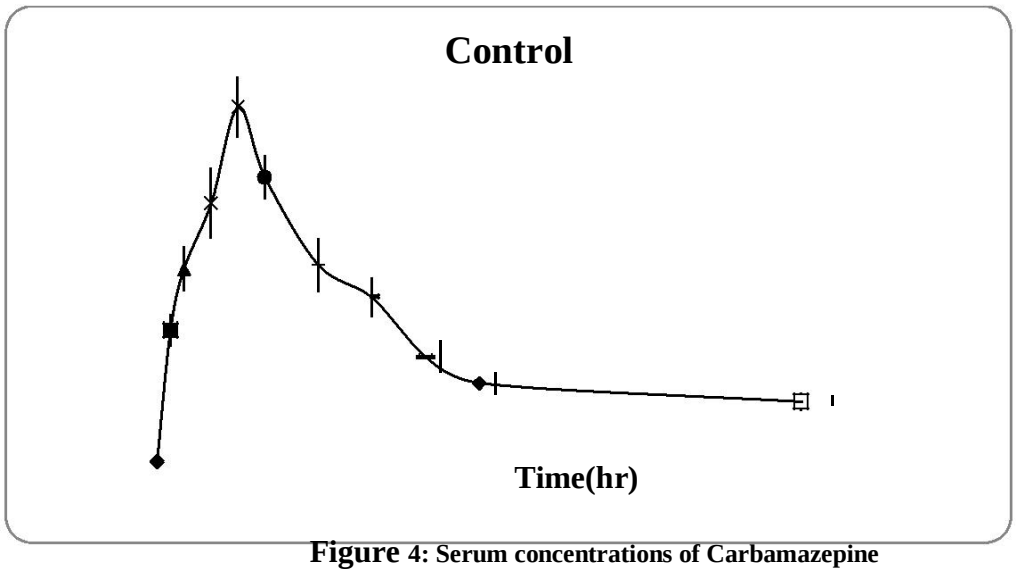

Figure 4: Serum concentrations of Carbamazepine

Parameter	Control
Cmax(µg/ml)	57.71±6.2
T max (hr)	3.00
T1/2 (hr)	12.89±1.5
Auc0-∞ (µg/ml/hr)	787.82±92
Vd(ml)	34.69±3.8
Cl(ml/min)	0.033±0.2
AUMC (µg/ml/hr^2)	19747.63±274.6 7
MRT (hr)	25.06 ±1.41

Table 2: Pharmacokinetic parameters of carbamazepine in rats

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Added con(µg/ml)	Recovered con(µg/ml)	S.D	% Recovery
0.00	0.00	0.00	0.00
3.12	3.16	0.22	103.34
6.25	6.36	0.75	101.77
12.50	12.15	0.81	95.18
25.00	24.22	0.69	97.19
50.00	49.16	1.19	95.83

Table 3: Inter day validation parameters (n=3)

Added con(µg/ml)	Recovered con(µg/ml)	S.D	% Recovery
0	0	0	0
3.13	3.09	0.14	97.96
6.25	6.14	0.71	95.36
12.50	11.91	0.71	93.18
25.00	25.22	0.71	104.49
50.00	52.16	2.12	101.47

Table 4: Intra-day validation parameters (n=3)

The recovered values were close to the true value suggesting the accuracy of the assay. The % recoveries of CBZ were ranged from 95.18 to 103.34% and 93.18% to 104.47% for inter-day and intraday, respectively. These high values of the % drug recovered reflect the accuracy of the assay method. The inter-day and intraday precisions were measured as the relative standard deviation (SD) expressed as percentage over the concentration range of CBZ during the course of validation. This is presented in Tables 1 and 2 for the inter-day and intraday precisions. The results indicated an acceptable precision for all concentrations assayed for both intraday and inter-day samples. The SD of CBZ ranged from 0.22 to 1.19 and 0.14 to 2.12 for both inter-day and intraday precisions, respectively. The low values of RSD% reflect the precision of the assay method. The LOQ for this method was found to be 0.5µg/ml. This indicated from the precision and accuracy of such concentration. Both accuracy and precision values throughout the concentration range (0.5–40 µg/ml) were acceptable (40). The specificity of the method with regard to CBZ was ascertained by the absence of any co-eluted or chromatographic interference peaks (Figs. 2 and 3).

IV. CONCLUSION

The HPLC system grew in this article is fast, touchy, and particular. The exactness and accuracy of the technique are inside the adequate extent (ICH Guideline Q2 (R1), 2005). The effortlessness of method and the high affectability make this procedure especially alluring for the measurement of CBZ in both arrangement and blood tests. The technique can likewise be promptly adjusted to routine quality control investigation.

REFERENCES

1. Duche P. Loiseau B. 1995. In: Levy R.H. Mattson R.H. Meldrum B.S. Penry J.K. Dreyfuss F.E. (Eds.) Antiepileptic Drugs fourth ed. Raven New York p. 555.
2. Goodman A.G. Hardman J.G. Limbrid L.E. 2001. Goodman and Gilman's the Pharmacological Basis of Therapeutics 10th ed. McGra w Hill New York.
3. He J. Shibukawa A. Nakagawa T. Direct injection analysis of carbamazepine and its active 10,11-epoxide metabolite in plasma by use of a

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 6, June 2015

semipermeable surface (SPS) silica column in LC. J. Pharm. Biomed. Anal VOL10, VOL 289–294, 1992.

4. Ashy A.R. El Sayed Y.M. Islam S.I. Comparison of fluorescence polarization immunoassay and high performance liquid chromatography for the quantitative determination ofphenytoinphenobarbitone and carbamazepine in serum. J. Pharm. Pharmacol, VOL 38 572 –577, 1986.

5. Hartley R. Lucock M. Cookman J.R. Becker M. Forsythe W.I. High-performance liquid-chromatographic determination of carbamazepine and carbamazepine-10,11-epoxide in plasma and saliva following solidphase sample extraction. J. Chromatogr, VOL 380 347 –356, 1986.

6. Chelberg R.D. Gunawan S. Treiman D.M. Simultaneous high-performance liquid-chromatographic determination of carbamazepine and its principal metabolites in human plasma and urine. Ther. Drug Monit, VOL 10 188–193, 1988.

7. Bhatti M.M. Hanson G.D. Schultz L. Simultaneous determination of phenytoin carbamazepine and 10,11-carbamazepine epoxide in human plasma by high-performance liquid
chromatography with ultraviolet detection. J. Pharm. Biomed. Anal, VOL 16 1233–1240, 1998.

8. Dasgupta A. Vega A.E. Wells A. Datta P. Effect of heating human sera at a temperature necessary to deactivate human immunodeficiency virus on measurement of free phenytoin free valproic acid and free carbamazepine concentrations. Ther. Drug Monit, VOL 21 421 –425, 1999.

9. Raggi M.A. Casamenti G. Mandrioli R. Sabbioni C. Volterra V. A rapid LC method for the identification and determination of CNS drugs in pharmaceutical formulations. J. Pharm. Biomed. Anal, VOL 23 161–167, 2000.

10. Franceschi L. Furlanut M. A simple method to monitor plasma concentration of oxcarbamazepine carbamazepine their main metabolites and lamotrignine in epileptic patients. Pharmacol. Res, VOL 51 297–302, 2005.

11. Oh E.K. Ban E. Woo J.S. Kim C.-K. Analysis of carbamazepine and its active metabolite carbamazepine-10,11- epoxide in human plasma using high-performance liquid chromatography. Anal. Bioanal. Chem, VOL 386 1931–1936, 2006.

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

13. Elizabeth G.-S. Darla R.L. Mohamed A.V. Matthew D.K. Simultaneous determination of lamotriginezonisamide and carbamazepine in human plasma by high-performance liquid chromatography. Biomed. Chromatogr, VOL 21 (3) 225–228, 2007.

14. Budakova L. Brozmanova H. Grundmann M. Fischer J. Simultaneous determination of antiepileptic drugs and their two active metabolites by HPLC. J. Sep. Sci, VOL 31 1–8, 2008.

15. Hemenway J.N. Jarho P. Henri J.T. Nair S.K. Velde D.V. Georg G.I. Stella V.J. Preparation and physicochemical characterization of a novel water-soluble prodrug of carbamazepine. J. Pharm. Sci, VOL 99 (4) 1810–1825, 2010.

16. Sanchez A. Garcia R. Abadin J.A. Duran J.A. Determination of free serum carbamazepine by protein precipitation with sulfosalicylic acid. Pharm. Pharmacol. Commun, VOL 5 435–438, 1999.

17. Lancas F.M. Sozza M.A. Queiroz M.E.C. Simultaneous plasma lamotrignine analysis with carbamazepine carbamazepine - 10,11-epoxide primidone phenytoin phenobarbital and PEMA by micellarelectrokinetic capillary chromatography (MECC). J. Anal. Toxicol, VOL 2 7 304–308, 2003.

18. Lee S.H. Li M. Suh J.K. Determination of carbamazepine by chemiluminescence detection using chemically prepared tris(2,2Vbipyridine) ruthenium(III) as oxidant. Anal. Sci, VOL 19 903–906, 2003.

19. Rezaei Z. Hemmateenejad B. Khabnadideh S. Gorgin M. Simultaneous spectrophotometric determination of carbamazepine and ph enytoin in serum by PLS regression and comparison Ruth HPLC. Talanta, VOL 65 21–28, 2005.

20. Escandar G.M. Gonza´ lez Go´ mez D. Espinosa Mansilla A. Mun˜ oz de la Pen˜ aA. Goicoechea H.C. Determination of carbamaz epine in serum and pharmaceutical preparations using immobilization on a nylon support and fluorescent detection. Anal. Chim. Acta, VOL 506 161–170, 2004.

21. Burke J.T. Thenot J.P. Determination of antiepileptic drugs. J. Chromatogr, VOL 340 199–241, 1985.

22. Owen A. Tettey J.N. Morgan P. Pirmonhamed M. Park B.K. LC determination of carbamazepine in murine brain. J. Pharm. Biomed. Anal, VOL 26 (4) 573–577, 2001.

23. Wad N.J. Simultaneous determination of eleven antiepileptic compounds in serum by high -performance liquid chromatography. J. Chromatogr, VOL 305 127–133, 1984.

24. Rouan M.C. Campestrini J. Le Clanche V. Lecaillon J.B. Godbillon J. Automated microanalysis of carbamazepine and its epoxide and trans-diol metabolites in plasma by column liquid chromatography. J. Chromatogr, VOL 573 (1) 65–68, 1992.

25. Juergens U. Routine determination of eight common antiepileptic drugs and metabolites by high -performance liquid chromatography using a column-switching system for direct injection of serum samples. J. Chromatogr, VOL 310 97–106, 1984.

26. Liu H. Delgado M. Forman L.J. Eggers C.M. Montoya J.L. Simultaneous determination of carbamazepine phenytoin phenobarbital primidone and their principal metabolites by high-performance liquid chromatography with photodiode-array detection. J. Chromatogr, VOL 616 105–115, 1993.

27. Huang C. He Q. Chen H. Flow injection photochemical spectrofluorimetry for the determination of carbamazepine in pharmaceutical preparations. J. Pharm. Biomed. Anal, VOL 30 (1) 59–65, 2002.

28. Chen K. Bashi H.K. Comparative analysis of antiepileptic drugs by gas chromatography using capillary or packed columns and by fluorescence polarization immunoassay. J. Anal. Toxicol, VOL 15 (2) 82–85, 1991.

29. Auer M.E. Griesser U.J. Sawatzki J. Qualitative and quantitative study of polymorphic forms in drug formulations by near infrared FT-Raman spectroscopy. J. Mol. Struct, VOL 661–662,
(1–3) 307–317 2003.

30. Mennickent S. Fierro R. Vega M. de Diego M. Godoy C.G. Instrumental planar chromatographic method for determination of ca rbamazepine in human serum. J. Sep. Sci,
VOL 32 (9) 1454–1458, 2009.

31. Queiroz R.H.C. Bertucci C. Malfara´ W.R. Dreossi S.A.C. Chaves A.R. Vale´ rio D.A.R. Queiroz M.E.C. Quantification of ca rbamazepine carbamazepine-10,11-epoxide phenytoin and phenobarbital in plasma samples by stir bar-sorptive extraction and liquid chromatography. J. Pharm. Biomed. Anal, VOL 48 428–434, 2008.

32. Patel R.B. Patel M.R. Bhatt K.K. Patel B.G. Development and validation of HPTLC method for estimation of carbamazepine in formulations and its in vitro release study. Chromatogr. Res. Int. 8pp 2011.

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 6, June 2015

33. Breton H. Cociglio M. Bressolle F. Peyriere H. Blayac J.P. Hillaire-Buys D. Liquid chromatography–electrospray mass spectrometry determination of carbamazepine oxcarbazepine and eight of their metabolites in human plasma. J. Chromatogr. B: Anal. Technol. Biomed. Life Sci, VOL 828 80–90, 2005.

34. Van Rooyen G.F. Badenhorst D. Swart K.J. Hundt H.K. Scanes T. Hundt A.F. 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, VOL 769 (1) 1–7, 2002.

35. Miao X.-S. Metcalfe C.D. Determination of carbamazepine and its metabolites in aqueous samples using liquid chromatography–electrospray tandem mass spectrometry. Anal. Chem, VOL 75 3731–3738, 2003.

36. Zhu Y. Chiang H. Wulster-Radcliffe M. Hilt R. Wong P. Kissinger C.B. Kissinger P.T. 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. VOL 38 (1) 119–125 2005.

37. AdukondaluDevandlaShravan Kumar Yamsani Y. MadhusudanRao. Rifampicin and Ketoconazole Modulation of Intestinal Transport of Carbamazepine in Rats 2013 Int J Pharm Sci Nanotech, VOL 6 (4) 10-13

38. Adukondalu D. Shravan Kumar Y. Vamshi Vishnu Y.Shiva Kumar R. MadhusudanRao Y 2010 Effect of pomegranate juice pre -treatment on the transport of carbamazepine across rat intestine Daru, VOL 18(4) 254-259

39. AdukondaludevandlaMadhusudanRaoYamsani Development and validation of an uplc method for the quantification of carbamazepi ne in intestinal sac samples 2015 Int J Pharm BiolSci, VOL5 (1) 145-152

40. ICH Guideline Q2 (R1) 2005 ICH Guideline Q2 (R1) 2005. Validation of Analytical Procedures: Text and Methodology.