IMPACT: International Journal of Research in Applied,
Natural and Social Sciences (IMPACT: IJRANSS)
ISSN (E): 2321-8851; ISSN (P): 2347-4580
Vol. 4, Issue 4, Apr 2016, 63-68

METHOD FOR OPTIMIZING HPLC FOR SEPARATING CARBAMAZEPINE AND IT'S IMPURITIES

ASHOK KUMAR, AMIT GUPTA, PRADEEP KUMAR, DEEPAK &. A.K.AGARWAL

Department of Chemistry N.R.E.C.College, Khurja, Bulandshahr, Uttar Pradesh, India

ABSTRACT

A simple, sensitive, precise and accurate HPLC method for simultaneous separation of Carbamazepine, Iminostilbene and Iminodibenzyl was developed and validated by using different stationary phases, mobile phases, and temperature parameters. The chromatographic separation of the drug and two impurities was achieved on a C_{18} column by using Water : Acetonitrile (60 : 40) as mobile phase, where a reasonable resolution, better sensitivity and a faster analysis time was obtained. Cyano column is used as the most suitable column for further optimization for keeping overall goal of a better separation and faster analysis.

KEYWORDS: Carbamazepine, Iminostilbene, Iminodibenzyl, HPLC

INTRODUCTION

Carbamazepine chemically, 5H-Dibenzo[b,f]azepine-5-carboxamide, is an anticonvulsant and mood-stabilizing drug used for treating epilepsy[(1)] and bipolar disorder. Two common impurities including Iminostilbene and Iminodibenzyl are associated with Carbamazepine. The structures of the drug and impurities are shown in figure 1-3.

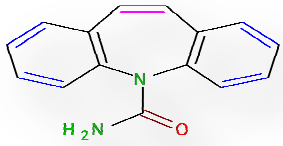

Figure 1: Carbamazepine

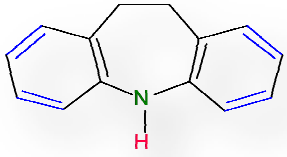

Figure 2: Iminodibenzyl

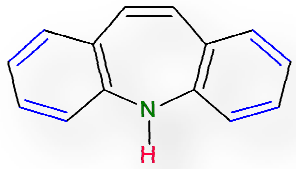

Figure 3: Iminostilbene

The drug is official in IP[2], BP[3] and USP[4]. Various methods used for determining these two impurities and other unknown impurities in Carbamazepine are TLC[5–10] and HPLC[11–13]. USP 23 method uses two different HPLC conditions for determining total impurities. One of the method mentioned which uses a C_{18} column and a mixture of Water, Acetonitrile and Methanol gives separation between the Carbamazepine and the two impurities mentioned above but takes a long time for elution of the iminodibenzyl peak (about 30 minutes). This results not only in higher run time but also in broadening of the peak resulting into a lower LOD and LOQ levels. Some optimization experiments focusing on the determination of Carbamazepine and other antiepileptic drugs have been reported[14–16]. We have separated the drug and the two impurities on a C_{18} column by using Water : Acetonitrile (60 : 40) as mobile phase. This method has been further optimized by changing column, mobile phase and temperature. A reasonable resolution, better sensitivity and a faster analysis time were obtained.

MATERIALS AND METHODS

- **Instrumentation**

A liquid chromatographic system from Shimadzu comprising autoinjector, quaternary gradient low–pressure pump and UV–visible variable wavelength detector connected to class VP software for controlling the instrumentation as well as processing the data generated, was used.

- **Reagents and Chemicals**

  - Acetonitrile and Methanol of HPLC grade were purchased from Ranbaxy Fine Chemicals Limited, New Delhi, India.
  - Reference standards of Carbamazepine, Iminostilbene and Iminodibenzyl were obtained from MAX India Ltd., and were used as it is without any further testing.

- **Chromatographic Condition**

Different mobile phases comprising different proportion of Water and Acetonitrile were prepared by mixing them in the desired ratios, filtering them through a 0.45 µ nylon membrane filter, and then degassing the filtered mobile phase by using sonnication. Three different columns from Macherey-Nagel comprising 3 different stationary phases viz. C_{18}, Phenyl and Cyano column (10µ, 25 cm × 4.0 mm ID) were used as stationary phase. Detection was carried out by using a UV detector at 254 nm, where injection volume was 10 µl.

- **Standard Preparation**

1000 ppm stock solutions for each of the two impurities were prepared by dissolving 50 mg of each of them in

chromatographic grade Methanol and diluting the resultant solution to 50 ml with the same solvent. 2 ml each of these two stock solutions were spiked into a 50 ml volumetric flask containing previously dispensed 100 mg of Carbamazepine. After dissolving the Carbamazepine with additional 5 ml of chromatographic grade Methanol, the whole solution was diluted upto the graduation mark of the 50 ml volumetric flask. The resultant solution has a concentration of 20 ppm of both the impurities and 2000 ppm of Carbamazepine.

- **Evaluation**

All the chromatograms generated during the optimization exercise were analysed by using the same integration parameters so as to make the comparison of the results obtained to be more meaningful. The parameters such as retention time, theoretical plates, resolution asymmetry and sensitivity of the peak were used to compare results obtained under various chromatographic conditions. The total run time required for the last peak to elute out was also considered as an important criterion. All the calculations were performed by using the software used for instrument control and data generation.

RESULTS AND DISCUSSIONS

Variation of Column

Three used phases were C_{18}, Phenyl and Cyano column with the same dimension (10µ, 25 cm × 4.0 mm ID) and from the same manufacturer namely Macherey Nagel, to make the comparison to be more meaningful. Mobile phase used in this exercise was a mixture of Water and Acetonitrile in a ratio of 60 : 40 (v/v). Chromatograms showing these separations are depicted in Figure 1A, 1B and 1C. The results obtained for the chromatographically important parameters are summarized in table 1.

Looking at the chromatograms and the relevant datas[17,18] it was found out that the C_{18} column gave the best separation between the Carbamazepine and both the impurities. However a higher run time was required. Phenyl column provided similar datas for the resolution, but the sensitivity was higher as represented by the height in µv, as compared to the C_{18} column. Cyano column gave lower resolution between the ISB and IDB peaks with very low retention times for the three peaks. Because of overall lesser run time, a better peak shape and highest sensitivity. Cyano column was considered as the most suitable column to be taken up for further optimization keeping in view the overall goal of a better separation, faster analysis and better sensitivity.

Variation of Mobile Phase

Using cyano column, analysis was carried out by preparing 2 additional mobile phases in the 65 : 35 and 70 : 30(v/v) ratio of Water and Acetonitrile. The chromatogram obtained are shown in figure 2 and the results obtained under the two conditions are tabulated in table 2.

The obtained results were studied in the same fashion as earlier.[19,20] There was not much difference in the theoretical plates count but the resolution between two closely eluting peaks viz. ISB and IDB was improved and a base line separation was achieved by using the mobile phase composition of 70 : 30 Water : Acetonitrile.

CONCLUSIONS

Variation of Temperature

In the above experiments, the analysis was carried out at 25°C. Using the cyano column and the optimized mobile phase of Water: Acetonitrile (70 : 30, v/v) a new experiment was carried out at a higher temperature of 35°C. A typical chromatogram of this separation is depicted in figure 3 and the results for the important chromatographic parameters are depicted in table 3. This provides a further improvement in sensitivity and helps to reduce the run time about 2 minutes without having any adverse effect on the separation as compared to the optimized condition of previous experiment.

REFERENCES

1. Merck Index, Eleventh Edition.
2. Indian Pharmacopeia 1996, p. 137.
3. British Pharmacopeia, 1993, p.108.
4. United State Pharmacopeia 23, 1995, p. 265.
5. Mehta, K.A., Kisaloglu, M.A., Phuaprdit, W., Malik, A.W., Shah, N.H. (2001). Int. J. Pharm. Sci., 217, 7−12.
6. Morkhade, M.M., Fulzele, S.V., Satturwar, P.M., Joshi, S.V., Guru Copal and Damar Gum (2006). Ind. J. Pharm. Sci., 68, 53−8.
7. Basak, S.C., Rahman, J., Ramalingam, M., Design, M. (2007). Pharmazie, 62, 145−8.
8. Gupta, K.R., Tajne, M.R., Wadokar, S.G. (2008). Ind. J. Pharm. Sci., 70, 511−3.
9. Kanna Rao, K.V., Rao, M.E., Nagozi, K.E., Rao, S.S. (2003). Ind. J. Pharm. Sci., 65, 204−6.
10. Selvameenal, L., Radha Krishna, M., Bala Gurunathan, R. (2009). Ind. J. Pharm. Sci., 71, 499−504.
11. Singh, R., Rao, H.S. (2008). Int. J. Green Chemistry, 2, 232−4.
12. Shaila, D.K., Kulkarni, S.M. (2006). Ind. J. Pharm. Sci., 68, 253−6.
13. Fang, L., Numagieu, S., Kobayashi, D., Ueda, H., Nakayama, K., Miyamo H. et.al., (2004). J. Pharm. Sci., 93, 144−54.
14. Guirgiis, M., Jammanli, F. (2001). J. Pharm. Sci., 4, 1077−84.
15. Hager, H.H., Medang, A.E., Eter, E.E., Arafa, M. (2007). Eur. J. Pharmacol., 5(54), 69−77.
16. Joharapurkar, A.A., Zanbad, S.P., Wanjari, M.M., Umathe, S.M. (2003). Ind. J. Pharmacol., 35, 236.
17. Sita Ram, C., Rupukanla, R.B., Reddy, B.N. and Sastry, S.P. (2011). Ind. Jour. of Pharm. Sci., 73(1) 107−110.
18. Morita, M.R., Berton, D., Boldvin, R., Barrows, F.A. (2008). E.C. Meur, J. Chromatogr. (B), 382, 132−9.

19. Quiming, N.C., Denola, N.L., Saito, Y., Chatabay, A.P., Jinn, K. (2008). Chromatographia, 67, 507.

20. Torres, S.Pous, Torres, J.R.F., Lapasio, Baeza, J.J., Garcia, M.C. (2009). Alvarezcoque, Anal. Bio. Anal. Chem., 394, 625.

APPENDIX

Table 1: Results of Optimisation of Column(A) C_{18} Column

Comp.	Rt (Min)	Plates	Resolution*	Asymmetry	Height µv
CARBA	4.80	1661	–	0.934	1909838
ISB	15.44	2083	11.66	0.89	86173
IDB	24.04	2203	5.06	0.88	5291

(B) Phenyl Column

Comp.	Rt (Min)	Plates	Resolution*	Asymmetry	Height µv
CARBA	5.35	3673	–	1.64	2398608
ISB	12.67	5354	14.00	1.51	166438
IDB	16.42	5337	4.71	2.24	11947

(C) Cyano Column

Comp.	Rt (Min)	Plates	Resolution*	Asymmetry	Height µv
CARBA	4.08	3675	–	1.30	2937296
ISB	7.11	5156	9.07	1.21	287174
IDB	8.20	5668	2.63	1.26	21686

Table 2: Results of Optimisation of Mobile Phase on Cyano Column (A) 65: 35 Water: acn (v/v)

Comp.	Rt (Min)	Plates	Resolution*	Asymmetry	Height µv
CARBA	4.53	3490	–	1.32	2892665
ISB	8.38	5224	4.16	1.25	263875
IDB	9.95	6122	3.23	1.02	20609

(B) 70: 30 Water: Acn (V/V)

Comp.	Rt (Min)	Plates	Resolution*	Asymmetry	Height µv
CARBA	5.10	3644	–	1.29	2728665
ISB	10.29	5502	4.31	1.28	211793
IDB	12.69	4940	3.75	1.25	23044

Table 3: Results of Optimisation of Cyano Column At 35°C 70: 30 Water: Acn (V/V)

Comp.	Rt (Min)	Plates	Resolution*	Asymmetry	Height µv
CARBA	4.87	3686	–	1.27	2868145
ISB	9.32	5478	4.11	1.23	238711
IDB	11.27	4888	3.39	1.23	25824