RESEARCH PAPER

Accepted 4 June 2005
Revised 20 January 2005
Received 8 July 2004
Indian J. Pharm. Sci., 2005, 67(3): 351-355

Validated High Performance Thin Layer Chromatography Method for Simultaneous Estimation of Phenytoin Sodium, Phenobarbitone Sodium and Carbamazepine in Tablet Dosage Forms

K. M. PATIL AND S. L. BODHANKAR*
Department of Pharmacology, Bharati Vidyapeeth Deemed University,
Poona College of Pharmacy, Erandwane, Pune-411038

A simple, specific, precise and rapid high performance thin layer chromatography method has been developed for estimation of phenytoin sodium, phenobarbitone sodium and carbamazepine simultaneously in tablet dosage form. In this method, standard solution and sample solution of phenytoin sodium, phenobarbitone sodium and carbamazepine were applied on precoated silica gel G60 F_{254} TLC plate and developed using a mixture of acetone:toluene (100:40 v/v) as mobile phase. The quantification was done by densitometry at 217 nm. This HPTLC system was quantitatively evaluated in terms of linearity, accuracy, precision, repeatability and specificity proving the utility in estimation of drug content in tablet dosage form.

Epilepsy is one of the most common neurologic disorders[1]. Phenytoin, phenobarbitone and carbamazepine are commonly used drugs in epilepsy population. Phenytoin is used to treat all types of seizure disorders except absence seizures, phenobarbitone is used to treat tonic clonic and partial seizures and carbamazepine is effective in treating seizures and bipolar disorder[2,3].

All the three drugs are official in IP[4], BP[5] and USP[6]. The assay procedures mentioned in IP and BP uses non-aqueous titration, gravimetric method and spectrophotometric method for the estimation of phenytoin sodium, phenobarbitone sodium and carbamazepine, respectively from tablet dosage forms. However in USP, a HPLC method is mentioned for the assay of all these three drugs. Also there are many reported HPLC, GC, SFC and immunological methods for the estimation of these drugs alone or simultaneously from pharmaceutical preparations[7-11] or biological fluids[12-16].

There is no reported HPTLC method for the estimation of these drugs alone or simultaneously in bulk and pharmaceutical preparation. The present study describes the development and validation of simple, specific, sensitive, accurate and precise HPTLC method for the determination of phenytoin sodium, phenobarbitone sodium and carbamazepine simultaneously in tablet dosage form.

MATERIAL AND METHODS

Phenytoin sodium and carbamazepine in pure powder forms were obtained as gift sample from Sun Pharmaceuticals Ltd., Mumbai. Phenobarbitone sodium was procured from B. J. Medical College, Pune. Silica Gel 60 F_{254} TLC plates (20x20 cm, layer thickness 0.2 mm, E-Merck, Darmstadt, Germany) were used as the stationary phase. Twenty tablets of Garoin® (phenytoin sodium-100 mg and phenobarbitone sodium-50 mg, Rhone Poulenc Ltd.) and Tegretol® (carbamazepine-200 mg, Novartis Pharmaceuticals Ltd.) were purchased from a local pharmacy. Toluene and acetone of AR grade purity were procured from Merck Ltd., Mumbai. Linomat IV sample applicator, twin trough developing chamber and TLC scanner III with CATS evaluation software (Version 4.06) were used in the studies (Camag, Muttenz, Switzerland).

*For correspondence
E. Mail: sbodh@yahoo.com

Standard and sample preparation:

Working standards of phenytoin sodium, phenobarbitone sodium and carbamazepine (10 mg each) were weighed accurately and diluted with methanol to obtain the final concentration of 100 µg/ml. Twenty tablets of Garoin® and Tegretol® were separately crushed and ground to a fine powder. A weight equivalent to 100 mg of phenytoin sodium, 50 mg of phenobarbitone sodium and 200 mg of carbamazepine was transferred to a conical flask and extracted with methanol. The extract was filtered through Whatman filter paper No. 1 and the residue was washed with sufficient amount of methanol. The extract and the washings were pooled, transferred to a 100 ml volumetric flask and the final volume was made upto 100 ml with methanol. Further 10 ml of filtrate was diluted to 100 ml to obtain the sample solution, which contains 100 µg/ml of phenytoin sodium, 50 µg/ml of phenobarbitone sodium and 200 µg/ml of carbamazepine.

HPTLC method and chromatographic conditions:

TLC plates were prewashed with methanol and dried in hot air. The chromatographic conditions maintained were precoated silica gel 60 F_{254} aluminum sheets (10×10 cm) as stationary phase, acetone: toluene in the ratio of 10:4 v/v as mobile phase, migration distance allowed was 80 mm, wavelength scanning was done at 217 nm keeping slit dimension of 5.0×0.45 mm. A deuterium lamp provided the source of radiation. Ten microlitres of standard solution (100 µg/ml mixture of each drug) was applied and developed at a constant temperature. Photometric measurements were performed at 217 nm in absorbance mode. Aliquotes of 5, 6, 7, 8, 9 and 10 µl of standard solution of phenytoin sodium, phenobarbitone sodium and carbamazepine were applied on the TLC plate (100 µg/ml of each drug). The TLC plate was dried, developed and analyzed photometrically.

Assay of tablet formulation:

Sample solution was spotted with volume 5 and 10 µl on to the TLC plate followed by development and scanning. The analysis was repeated in triplicate. The spot was resolved into three peaks in the chromatogram of drug samples, extracted from the tablet formulation. The content of the drug was calculated from the peak areas recorded.

Method validation:

The method was validated as per ICH guidelines[17] in terms of linearity, accuracy, inter-day and intra-day precision, reproducibility of measurement of peak area, reproducibility of sample application and specificity. The limit of quantification and limit of detection for phenytoin sodium, phenobarbitone sodium and carbamazepine were also determined. Accuracy of the analysis was evaluated by carrying out a recovery study. For that purpose known concentration of standard drug was added to a preanalysed tablet sample at three different levels namely 80, 100 and 120% and average recovery was calculated.

The intra-day precision was determined by analyzing standard drug solutions in the concentration range of 500 to 1000 ng/spot for 3 times on the same day, while inter-day precision was determined by analyzing corresponding standards daily for a period of one week. Repeatability of measurement of peak area was determined by spotting 10 µl of standard drug solution on a TLC plate and developing the plate. The separated spot was scanned 7 times without changing position of plates and relative standard deviation (RSD or % CV) for measurement of peak area was calculated. Repeatability of sample application was assessed by spotting 10 µl of standard drug solution 7 times on a TLC plate by semiautomatic spotter, followed by development of plate and recording the peak areas for seven spots. The RSD for the peak area values was calculated.

The specificity of the proposed method was checked by spotting a sample solution of phenytoin sodium, phenobarbitone sodium and carbamazepine on the TLC plate and developing and scanning the plate as described earlier. Purity was also checked by overlaying the spectra of standard phenytoin sodium, phenobarbitone sodium and carbamazepine solution with the spectra of sample recorded on a TLC scanner in UV range.

RESULTS AND DISCUSSION

Various methods have been reported for analysis of phenytoin sodium, phenobarbitone sodium and carbamazepine alone or simultaneously in biological fluids or pharmaceutical preparations. Most of them are HPLC, GC or immunological methods, which are sophisticated, costly and time consuming. The developed method may turn out to be easy and cost effective for the routine analysis purposes such as assay and determination of content uniformity. Therefore, it was decided to develop a method of analysis of these drugs using HPTLC, a versatile speedy and cost effective technique.

The solvent system having a combination of toluene and acetone (10:4) offered maximum resolution for all the three drugs with R_f values of 0.21 (±0.03), 0.45 (±0.02) and

0.54 (±0.02) for carbamazepine, phenytoin sodium and phenobarbitone sodium, respectively (fig. 1). After development, the plate was scanned at wavelength 217 nm, as all the three antiepileptic drugs presently assayed had optimum absorption at or around this band (fig. 2)

Since these drugs are freely soluble in methanol, the tablet powder was extracted with methanol. Sonication for 10 min helped to extract them completely from tablet matrix. The amount of antiepileptic drugs in tablet formulation was calculated on applying suitable dilution factor and comparing peak area of the standard and sample solutions. The assay of phenytoin sodium, phenobarbitone sodium and carbamazepine in tablet formulation calculated as per peak area was found to be 100.2±0.61, 100.4±0.67 and 99.8±0.42 %, respectively (Table 1).

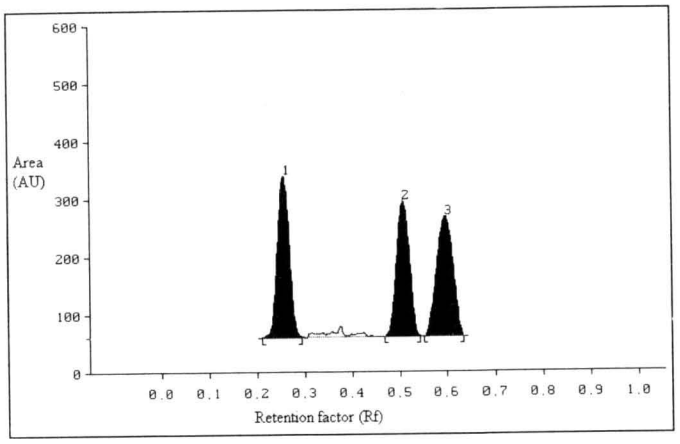

Fig. 1: Typical densitogram of carbamazepine, phenytoin sodium and phenobarbitone sodium.

Peak 1, 2 and 3 are carbamazepine, phenytoin sodium and phenobarbitone sodium, respectively.

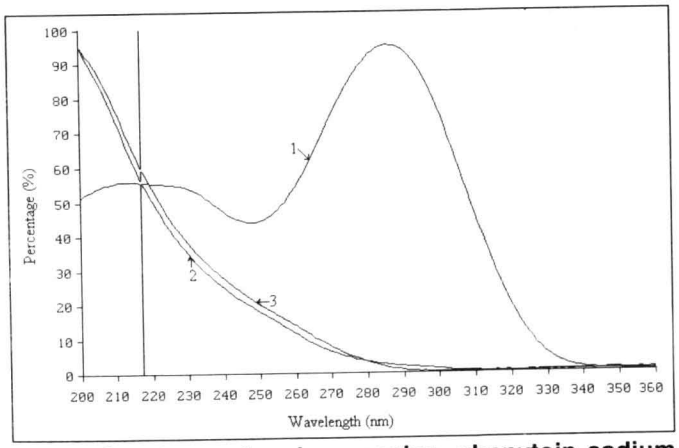

Fig. 2: Spectrum of carbamazepine, phenytoin sodium and phenobarbitone sodium.

Spectrum 1, 2 and 3 are carbamazepine, phenytoin sodium and phenobarbitone sodium, respectively.

TABLE 1: ASSAY OF PHENYTOIN SODIUM, PHENOBARBITONE SODIUM AND CARBAMAZEPINE

Labeled claim	Amount found ± SD (mg)*	%Assay ± SD*	%CV
Phenytoin sodium- 100 mg	100.2±0.61	100.2±0.61	0.609
Phenobarbitone sodium- 50 mg	50.2±0.33	100.4±0.67	0.675
Carbamazepine - 200 mg	199.6±0.85	99.8±0.42	0.428

*Average value±standard deviation of five determinations

The good average recovery values obtained in recovery studies indicate that the proposed method is accurate for estimation of drug in tablets (Table 2). The intra-day and inter-day coefficient for all three drugs was found to be in the range of 1.75 to 3.61 % and 1.98 to 5.17 %, respectively. Lower values of intra-day and inter-day variation in the analysis indicate that the method is precise. The RSD for repeatability of measurement of peak area and RSD for repeatability of sample application were found well below the instrumental specifications, ensuring proper functioning of HPTLC system. It was observed that excipients present in formulation did not interfere with peaks of carbamazepine (R_f=0.21±0.03), phenytoin sodium (R_f=0.45±0.02) and phenobarbitone sodium (R_f=0.54±0.02). The purity was confirmed by overlaying the spectra of standard mixture of drugs with the spectra of sample recorded on TLC scanner in UV range, which shows the specificity of method. Different validation parameters for the proposed HPTLC method for determination for phenytoin sodium, phenobarbitone sodium and carbamazepine have been summarized in Table 3.

The proposed HPTLC method was found to be rapid, cheaper, simple, specific, sensitive, precise and accurate. Thus it can be employed for the routine quality control analysis of phenytoin sodium, phenobarbitone sodium and

TABLE 2: RECOVERY OF PHENYTOIN SODIUM, PHENOBARBITONE SODIUM AND CARBAMAZEPINE

Label claim (mg/tablet)	Amount added (%)	Total amount added (mg)	Amount Recovered* (mg)	% Recovery*	Average Recovery (%)
Phenytoin sodium-	80	80	81.0±0.33	101.3±0.42	101.2 ± 0.40
100	100	100	100.2±0.31	100.2±0.31	
	120	120	122.4±0.58	102.0±0.48	
Phenobarbitone	80	40	40.6±0.50	101.6±1.26	100.8±1.35
sodium-	100	50	50.2±0.56	100.5±1.13	
50	120	60	60.2±1.0	100.3±1.66	
Carbamazepine-	80	160	157.9±0.52	98.7±0.32	98.8±0.41
200	100	200	197.1±1.15	98.6±0.57	
	120	240	238.4±0.78	99.3±0.32	

*Average value±standard deviation of five determinations

TABLE 3: METHOD VALIDATION PARAMETERS

Parameter	Result		
	PHT	PB	CA
Linearity range (ng/spot)	500-1000	500-1000	500-1100
Correlation coefficient (r)	0.9898	0.9865	0.9965
Limit of detection (LOD)	40 µg/ml	40 µg/ml	20 µg/ml
Limit of quantification (LOQ)	90 µg/ml	90 µg/ml	40 µg/ml
Accuracy	101.2 %	100.8 %	98.8 %
Precision (%CV)			
Repeatability of application (n=7)	0.83	0.85	0.55
Repeatability of measurement (n=7)	0.36	0.34	0.21
Intra-day (n=3)	2.0-3.41	2.82-3.61	1.75-2.56
Inter-day (n=3)	3.24-4.85	3.60-5.17	1.98-3.44
Specificity	Specific	Specific	Specific

carbamazepine from tablets or other dosage forms.

ACKNOWLEDGEMENTS

The authors wish to acknowledge with thanks to Principal. Dr. S. S. Kadam and Vice Principal, Dr. K. R. Mahadik, Poona College of Pharmacy, Pune for encouragement and support for our study. Author thanks M/s Sun Pharmaceuticals Limited for providing pure working standard of phenytoin sodium and carbamazepine. Also thanks are due to the Department of Pharmacology, B. J. Medical college for providing pure working standard of phenobarbitone sodium.

REFERENCES

1. Bazil, M.K and Bazil, C.W., **Clin. Ther.**, 1997, 19, 369.
2. Warner, A., Privitera, M and Bates, D., **Clin. Chem.**, 1998, 44, 1085.
3. Tripathi, K.D., Eds., In; Essentials of Medical Pharmacology, 4th Edn., Jaypee Brothers Medical Publishers (P) Ltd., New Delhi, 1999, 381.
4. Indian Pharmacopoeia, 4th Edn., The controller of publications, New Delhi, 1996, 137.
5. British Pharmacopoeia, 15th Edn., British Pharmacopoeia Commission, Health Ministry, London, 1993, 108.
6. United States Pharmacopoeia, 24th Edn. The United States Pharmacopoeial Convention, Inc., Rockville, MD, 2000, 301.

7. Vio, L., Mamolo, M.G and Furlan, G., **Farmaco,** 1988, 43, 157.
8. Wahbi, A.M and Bararay, M., **Analyst,** 1980, 105, 855-60.
9. Cyr, T.D., Matsui, F., Sears, R.W., Curran, N.M and Lovering, E.G., **J. Assoc. Off. Anal. Chem.,** 1987, 70, 836.
10. Mamolo, M.G., Vio, L and Maurich, V., **Boll. Chim. Farm.,** 1984, 123, 591.
11. Patil, S.T., Bhoir, I.C and Sudaresan, M., **Analytica. Chemica. Acta.,** 1999, 384, 143.
12. Johannessen, S.I., In; Levy, R.H., Mattson, R.H and Meldrum, B.S., Eds., Antiepileptic drugs, 4th Edn., Raven Press, Ltd, New York, 1995, 243.
13. Bhatti, M.M., Hanson, G.D and Schultz, L., **J. Pharm. Biomed. Analysis.,** 1998, 16, 1233.
14. Matar, K.M., Nicholls, P.J., Tekle, A., Bawazir, S.A and Al-Hassan, M.I,. **Ther. Drug. Monit.,** 1999, 21, 559.
15. Kishore, P., Rajnarayana, K., Reddy, M.S., Sagar, J.V and Krishna D.R., **Arzeim-Forsch-Drug Res.,** 2003, 53, 763.
16. Levert, H., Odou, P and Robert, H., **Biomed. Chromatogr.,** 2002, 16, 19.
17. ICH Guideline Q2B, Validation of Analytical Procedure: Methodology, Geneva, 1996, 1.