ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
28 (2002) 789–793

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Short communication

Determination of lacidipine from urine by HPTLC using off-line SPE

V.R. Kharat [a,*], K.K. Verma [b], J.D. Dhake [a]

[a] *Department of Chemical Technology, Laxminarayan Institute of Technology, University of Nagpur, Nagpur 440010, India*
[b] *Department of Chemistry, Rani Durgavati University, Jabalpur 482001, India*

Received 27 September 2000; received in revised form 28 February 2001; accepted 22 July 2001

Abstract

A simple, rapid and sensitive high performance thin layer chromatographic method was developed and validated for the estimation of lacidipine. The sample preparation involved protein precipitation followed by an efficient solid phase extraction on C18 cartridge. The analytes were isolated from 1 ml of urine and recovered by pure ethyl acetate solution. The method employed TLC aluminium plate precoated with silica gel 60F254 as the stationary phase. The solvent system employed consists of toulene–ethyl acetate [6.5:3.5v/v]. This system gave a dense and compact spot of the drug at R_f value of 0.45. The linear regression data for the calibration plots showed good linear relationship ($r = 0.999$) over the concentration range 10–80 ng. Recovery studies were performed at two different levels. The recovery data reveals that the R.S.D. for intra-day and inter-day analysis at 10 ng was found to be 0.84 and 0.22%, respectively. The proposed method was found to be useful for the routine analysis of pharmaceuticals and pharmacokinetic studies in human urine samples. © 2002 Published by Elsevier Science B.V.

Keywords: HPTLC; Pharmacokinetic studies; Urine

1. Introduction

Lacidipine is a dihidropyridine class of a drug and is widely used as a calcium channel blocker in the treatment of hyertention. It is administered at very low doses.

Lacidipine is official in Martindales extra pharmacoepia [1]. Literature survey reveals variety of analytical methods for the determination in pharmaceutical dosage forms, as well as in biological fluids. The powerful hyphenation of liquid chromatography and mass spectrometry is used to detect and assay the lacidipine in biological fluids [6,7]. Also liquid chromatographic systems [3], radioimmunoassay [4,5], and spectrophotometry [2] have been developed. Although these methods have their own advantages, they are costly. Recently lacidipine has been determined by HPTLC in pharmaceutical preparations in this laboratory. Liquid–liquid extractions [2,5–7] as a sample clean up procedures are frequently used for the determination of lacidipine from urine and pharmaceutical preparations. These methods lacks in

* Corresponding author.
E-mail address: vishakhkharat@rediffmail.com (V.R. Kharat).

0731-7085/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S0731-7085(01)00584-2

removing interfering substances. Although aspect system with RIA has been established with higher sensitivity in plasma, it is time consuming and costly for this purpose we established a simple and cost effective sample clean up procedure using solute seppak C-18 cartridges (100 mg/ml) with hypodermic syringe for the analysis of lacidipine in human urine samples using HPTLC with ultra violet (UV) detection.

Over past decade, high performance thin layer chromatography (HPLC) has been successfully used in the pharmaceuticals. Here we describe simple, sensitive, accurate and precise analytical procedure for the determination of lacidipine using simple HPTLC method with UV detection. After efficient purification by SPE on isolute seppak C-18 cartridges, this procedure was used to determine lacidipine using HPTLC with UV detection. The added advantage of this method is large number of samples can be simultaneously analysed.

The solid phase extraction has various advantages over that of liquid–liquid extraction with regard to convenience, simplicity and rapidity.

The proposed method requires less quantities of solvents and simple and inexpensive solid phase extraction assembly.

2. Experimental

2.1. Materials

Lacidipine (purity 99.98) was a gift from Sun Pharmaceuticals Ltd India. Chromatograhic grade ethyl acetate and toluene were procured from S.D. Fine Chemicals Ltd India.

Solid phase extraction was performed using Isolute C18 cartridges (100 mg/1 ml).

2.2. Instrumentation

A camag linomat IV automatic sample applicator was used to dispense an aliquots(10 μl) of lacidipine on to the aluminium backed silica 60F254 (format, 10/10 cm having thickness of 250 μm, E. Merck Germany) as 6 mm bands. The plates were developed with toulene–ethyl acetate (6.5:3.5 v/v) solvent system using linear ascending technique in a Camag twin trough chamber. The chamber saturation time for the mobile phase was optimised to 10 min to ensure the concentrated zone of the compound and hence better resolution. The length of chromatogram run was set at 7 cm. After development, the TLC plates were dried in a current of using an air blower. Evaluation was performed with Camag TLC scanner III densitometer controlled by CATS software version 4.06, absorbance was measured at 287 nm (figure 2) using deuterium lamp. Peak heights were recorded for all the tracks.

2.3. Solid phase extraction

Urine samples are often used to obtain pharmacokinetic information. To enable the direct, simple and cost effective determination of lacidipine without derivatisation, purification process prior to their determination was based on SPE. SPE technique in biological samples would be very useful in carrying out clinical test.

Initially the SPE method was optimised. Initial trials for eluting the solute (without urine matrix and lacidipine could retain on cartridges) were taken by using various solvents i.e. methanol, ethyl acetate, ethanol. Ethyl acetate shows best results, since lacidipine is readily soluble in it. Trials were also conducted for elution without adding acetonitrile to urine sample, it has been observed that it takes more time for elution.

To 1 ml blank urine, a definite volume of methanolic solution containing 10–80 ng of lacidipine was spiked. Then, 1 ml of acetonitrile was added to precipitate proteins, since proteins block the cartridges. After vortex mixing for 15 s, all samples were centrifuged at $1500 \times g$ for 10 min. The supernatant was then transferred to extraction cartridges (Isolute seppak C 18) which were successively conditioned twice with 2 ml of methanol and twice with 2 ml of water. Pretreated calibration samples or standards were allowed to drain through the cartridges and left to dry for 1 min.

Extraction cartridges were washed twice with 2 ml water. The analytes were then eluted with the four volumes of 500 μl ethyl acetate. Ethyl acetate

was then evaporated to dryness under a stream of nitrogen at 40 °C and re dissolved 200 μl of methanol and the solution was spotted on the plate and developed as described in Section 2.2. The peak height was recorded.

2.4. Calibration plots

A series of standard solution were prepared over a range of 10–80 ng from a stock solution of lacidipine (1 mg/ml). The procedure for sample preparation was followed as described in Section 2.3.

A calibration cure was constructed for lacidipine by plotting the peak height [Y-axis] against the amount of lacidipine [X-axis].

2.5. Solution stability

The solution was stable throughout the analysis. The spot was developed after3, 6, 9 and 12 h after the solution was prepared, no secondary pot was observed. The low coefficient of variation (COV) and a single spot shows the stability of the solute in the solution.

2.6. Method validation

Reproducibility and recovery studies were performed in a view to justify the accuracy, suitability and precision of the proposed method. The concentration with linearity range (10 and 60 ng) were selected and analysed for five times. The intra-day and inter-day variabilities are determined for the compound in a series of consisting of five spiked urine samples. In order to estimate the limit of detection, solution of compound in methanol was used and the limit of quantitation was evaluated from five extracted urine samples.

Recoveries of lacidipine were determined by the comparison of the peak height obtained from the processed urine sample with the peak height of the directly spotted standards. Urine samples with known concentration of 10 and 60 ng, as well as standard solutions in methanol were used. No internal standard was used. The analysis was also done by other analyst and the method showed the good ruggedness. Since we are having only one instrument robustness could not be determine.

3. Results and discussion

3.1. Optimisation of chromatogram

Initial trial experiments were conducted in a view to select a suitable solvent system for the accurate estimation of the drug. Toulene–ethyl acetate in a varying ratios (1:1, 6:4, 6.5:3.5, 7:3, 8:2) were tried. But a 6.5:3.5 and 7:3 gave a dense and compact spot. Finally a solvent system of toulene–ethyl acetate (6.5:3.5 v/v)was selected as the optimum mobile phase for the development of chromatogram. The solvent system gave a dense and compact spot on the plate and well resolved peak with R_f value of 0.45($n = 5$). A typical densitogram is depicted in Fig. 1.

3.2. Calibration plots

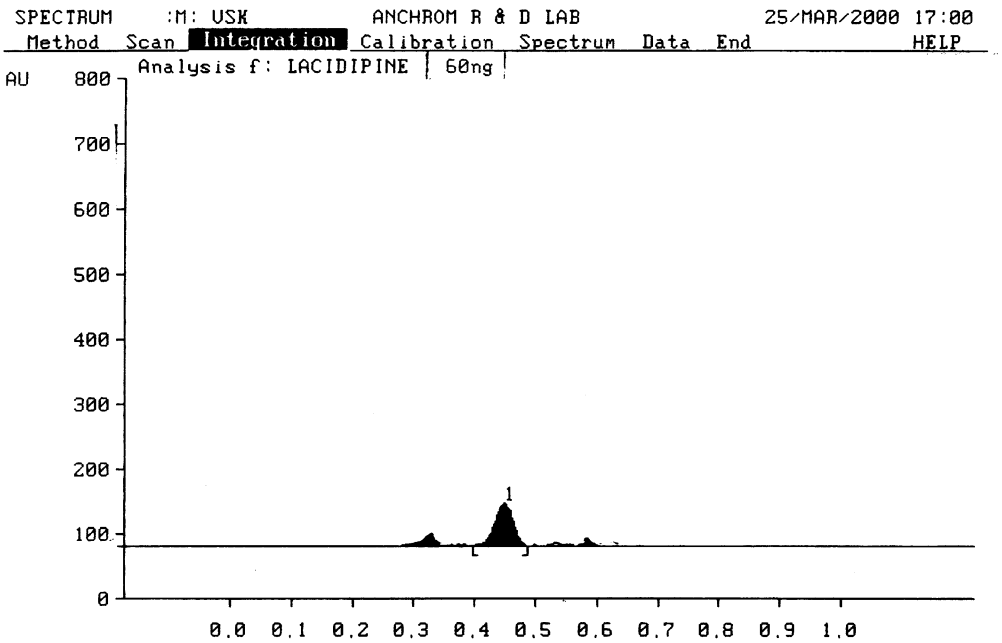
The peak height versus drug concentration was plotted to construct a standard curve of lacidipine. The Linear regression for the calibration plots showed a good linear relationship with the coefficient of correlation $r = 0.999$, slope $= 0.9386$, and intercept $= 2.0314$ ($n = 5$) over the concentration range studied. The range of reliable quantification was set at 10–80 ng. The R.S.D. values for slope and intercept has been found to be 0.0001 and 0.0053%, respectively.

3.3. Limit of detection and limit of quantification

The limit of detection of lacidipine has been obtained by reducing the drug concentration and the value obtained was 5 ng and the limit of quantification has been to be 8 ng.

3.4. Validation

The results of precision and accuracy of the method for lacidipine are given in Tables 1 and 2. The results show the good precision of the method for the determination of the lacidipine from the biological samples. The intra-day and inter-day COVs for the assay is less than 1% at lower concentration and less than 1% higher concentration also.



Peak	Start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.40	0.00	0.45	64.9	100.00	0.49	0.0	1262.4	100.00

Total height = 64.9 Total area : 1262.4

Fig. 1.

Table 1
Validation of the method

	Concentration spiked (ng)	Concentration found (ng)[a]	COV (%)
Inter-day	10	8.75 ± 0.02	0.22
	60	59.70 ± 0.21	0.35
Intra-day	10	8.85 ± 0.075	0.84
	60	61.07 ± 0.35	0.57

[a] Mean ± standard deviation (S.D.) $n = 5$.

Table 2
Extraction recoveries of lacidipine ($n = 5$)

Compound	Concentration (ng)	Mean peak height (std.)	Mean peak height (ext.)	Extraction recoveries[a]
Lacidipine	10	10.9	10.35	95% ± 0.67
	60	65.8	59.33	90% ± 0.49

[a] Mean ± S.D. ($n = 5$).

The extraction recoveries of lacidipine are shown in Table 2, Recoveries are higher than 95% at lower concentration (i.e. 10 ng) and higher than 90% at higher concentration (i.e. 60 ng).

3.5. Specificity

The method is specific for lacidipine only. Since no secondary spot was observed, there was no any interfering substance on the plate.

4. Conclusions

The method described is sensitive particularly at lower concentrations, rapid and single step solid phase extraction. Since many samples can be analysed within a short time, the method can be used for the routine analysis in a quality control laboratory and the determination of the drug from the biological fluids i.e. plasma, serum etc. The method is also useful for the pharmacokinetic studies of drug and clinical tests.

Acknowledgements

The authors are thankful to Sun Pharmaceuticals Ltd for providing gift sample of lacidipine.

References

[1] Extra Pharmacoepia of Martindale 31st ed. 1999, pp. 898.
[2] S.N. Meyyanathan, G.V.S. Rama Sarma, B. Suresh, Indian Drugs 36 (1999) 9.
[3] R. Beiyuan, Z. Binquang, L. Zuqing, Zongguo yaoxuehui Zazhi, Beiging 32 (12) (1997) 764–766.
[4] S. Braggio, S. Serter, F. Angeri, J. Chromatogr. Biomed. 669 (2) (1995) 382–389.
[5] S. Lisa, C. Ggion Elisabeth, S. Braggio, M. Pellegalli, J. Cardiovasc. Pharmacol. (Suppl. 5) (1994) S94–S97.
[6] P. Rosseti, M. Scandola, P. Grossi, J. Chromatogr. 647 (1) (1993) 155–156.
[7] P. Rossato, M. Scandola, F. Pughnagh, P. Grossi, Org. Mass Spectrum. 27 (11) (1992) 1261–1265.