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Simultaneous determination of lignocaine hydrochloride and phenylephrine hydrochloride by HPTLC

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Abstract

A high performance thin layer chromatographic (HPTLC) method for the simultaneous quantification of lignocaine hydrochloride (LIG) and phenylephrine hydrochloride (PHE) is described. The mobile phase consisted of ethyl acetate–methanol–ammonia (4:1:0.4 v/v/v). The densitometric determination of LIG and PHE was carried out at 262 nm and 291 nm, respectively. The calibration curves of LIG and PHE were linear in the range of 8–18 μg and 4–9 μg, respectively. The method was validated with respect to system precision, method precision, recoveries, intra-day and inter-day variation. The system was applied for the simultaneous determination of LIG and PHE from a new drug delivery system. The results indicate that the method is simple, specific, selective and reliable for simultaneous quantitative determination of LIG and PHE as bulk drug and from formulations.

Keywords: Simultaneous; Lignocaine hydrochloride; Phenylephrine hydrochloride; HPTLC

1. Introduction

Lignocaine hydrochloride (LIG) is a widely used local anaesthetic in dentistry [1,2]. Local anaesthetic drugs being vasodilators are often combined with vasoconstrictor agents. Vasoconstrictors reduce systemic absorption and post-operative bleeding and prolong the duration of action of local anaesthetic [1]. Phenylephrine hydrochloride (PHE), a nonspecific sympathomimetic agent, when administered along with local anaesthetics produces clinically useful prolongation in anaesthesia [3]. LIG and PHE could be a useful drug combination for the design of non-invasive Drug Delivery System (DDS) for local anaesthesia in dentistry.

LIG [4] and PHE [5] exhibit absorption maxima at 262 nm and 273 nm. The U.S.P and B.P describe titrimetric methods for the assay of LIG [6,7] and PHE [8,9]. A number of LC and GC procedures have been described for LIG [10–17] and PHE [18–23]. However, they are often time consuming, expensive and laborious.

Over the past decade, high performance thin layer chromatography (HPTLC) [24–28] has been successfully used in the analysis of pharmaceuti-

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cals, plant constituents and biomacromolecules. Solvent consumption per sample basis with HPTLC is only 5% of that with LC, thus this method becomes more economical and less hazardous. A number of samples can be analysed in a single run, reducing the analysis time. HPTLC allows visualisation of samples at every stage of analysis, moreover post chromatographic derivatisation is easy. Hence, the present study was undertaken to establish a simple, rapid and yet very specific and selective technique for quantitative determination of LIG and PHE separately and in combination for routine analysis.

2. Experimental

2.1. Chemicals and reagents

LIG and PHE were received as gift samples from Gufic (Mumbai, India), and Wallace Pharmaceuticals (Mumbai, India), respectively. Chromatographic grade solvents were purchased from Ranbaxy Chemicals (Delhi, India). All the reagents were of analytical grade and were used without further purification.

2.2. Instrumentation

LIG and PHE were spotted as narrow bands of 5mm width at a constant rate of 15 s/μl on precoated silica gel $60F_{254}$ plates (layer thickness 250 μm) using a Camag Linomat IV model, under a nitrogen atmosphere. The mobile phase consisting of ethyl acetate–methanol–ammonia (4:1:0.4 v/v/v) was used for method development. The chamber was saturated with the mobile phase for 30 min. The length of the chromatographic run was 8 cm and the time required for each run was $\approx$ 20 min. The separation was visualized by irradiation of the plates with 254 nm. Densitometric analysis of the separated components was carried out using the Camag TLC scanner II 1988 model in the absorbance mode at 262 nm and 291 nm, respectively. The scanning speed was kept at 4 mm/s. Chromatograms were integrated on a Perkin Elmer integrator system LCI-100.

2.3. Assay procedure

2.3.1. Standard curve of LIG in water

A total of 50 mg of LIG was dissolved in 50 ml of distilled water to obtain stock solution of LIG (1 μg/μl). Appropriate quantities of this stock solution were spotted to obtain LIG concentration in the range of 8–18 μg.

2.3.2. Standard curve of PHE

Twenty miligrams of PHE was dissolved in 50 ml of distilled water to obtain a stock solution of PHE (0.5 μg/μl). Appropriate quantities were spotted to obtain PHE concentration in the range of 4–9 μg.

2.3.3. Standard curve of mixture of LIG and PHE

A combined stock solution of LIG(1 μg/μl) and PHE (0.5 μg/μl) was prepared in distilled water. Appropriate quantities of this stock solution were spotted to obtain concentrations of 8–18 and 4–9 μg of LIG and PHE, respectively. Series of concentration of LIG and PHE were spotted and analysed to determine the lowest concentration that can be accurately detected and integrated.

2.3.4. Precision studies

Six bands were applied from a single stock solution (LIG-14 μg and PHE-7 μg) on silica gel 60 F_{254} plates and analysed by the proposed method for system precision studies to determine variations due to the instrument and six different samples of the same concentration (LIG-14 μg and PHE-7 μg) were spotted on silica gel 60 F_{254} plates and analysed by the proposed method to determine variations arising due to the method itself.

2.3.5. Ruggedness studies

The standard curves of LIG and PHE prepared individually and in combination were evaluated for within-day ($n = 3$) and week-to-week ($n = 3$) reproducibility.

2.3.6. Solution stability of LIG and PHE during the analysis

Samples were spotted from a stock solution at the time interval of 30, 60, 90 and 120 min after sample preparation and analysed by the proposed method ($n = 3$).

2.3.7. recovery studies from new drug delivery system (DDS)

The study was performed at both the lower and higher concentrations of LIG and PHE. A recovery study was carried out by adding known amounts of the drug (solution of drug from DDS) to obtain a concentration of 12 and 16 μg for LIG and 5 and 8 μg for PHE. The resulting mixtures were analysed by HPTLC.

2.3.8. Analysis of LIG and PHE from DDS

The drug delivery system(DDS) was a polymeric system containing 20 mg LIG and 0.5 mg PHE. Ten such DDS were dissolved in distilled water. The solution was filtered through a G4 sintered funnel and the volume made to 50 ml with distilled water. Aliquots of 3 and 50 μl of the solution were spotted to analyze LIG and PHE content, respectively using the developed HPTLC method.

3. Results and discussion

3.1. Standardization of chromatographic conditions

Various solvent systems were evaluated to arrive at an optimum resolution of the two drugs. The solvent system consisting of ethyl acetate–methanol–ammonia (4:1:0.4) gave dense, compact and well separated spots of the drugs from the mixture. The R_f values were found to be 0.75 and 0.23 for LIG and PHE, respectively. Ammonia in the mobile phase liberates lignocaine and phenylephrine from their hydrochloride salts. Phenylephrine has a maximum at 291 nm as compared to its hydrochloride salt, which shows an absorption maximum at 273 nm [29]. Hence densitometric analysis of LIG and PHE was performed at 262 and 291 nm, respectively. Adequate separation of the two drugs enabled the development of a selective and specific method of analysis.

Table 1
Standard curve data for LIG and PHE[a]

Drug	Intercept	Slope	r
LIG	22594.00 ± 553	27.3698 ± 0.434	0.992 ± 0.001
LIG in combination	22592.89 ± 550	27.3699 ± 0.382	0.992 ± 0.001
PHE	18528.35 ± 255	60.6113 ± 0.580	0.995 ± 0.003
PHE in combination	18521.47 ± 246	60.6091 ± 0.573	0.995 ± 0.001

[a] $n = 6$.

3.2. Standard curves of LIG and PHE

A series of standard curves ($n = 6$) each of LIG and PHE were prepared over a concentration range of 8–18 and 4–9 μg, respectively, separately and in combination. The standard curves were linear over the range examined. The mean values of intercept, slope and correlation coefficient are shown in Table 1. The limit of reliable quantification was set at 8–18 and 4–9 μg for LIG and PHE, respectively, as no significant difference was observed in the slopes of the standard curves in this range (ANOVA; $P > 0.05$).

The detection limit of LIG and PHE were found to be 4 and 2 μg, respectively. These were the lowest concentration of drugs that were accurately detected and integrated by the instrumentation used. Below this concentration, the spots were not clearly visible. The coefficient of variation was found to be 2.1% ($n = 6$) and 1.4% ($n = 6$) and no noise was observed.

3.3. Precision studies

As depicted in Table 2 relative standard deviation of less than 2% suggests system suitability and precision of the developed method.

Table 2
System precision and method precision studies of the developed method

No.	System precision		Method precision	
	Area LIG	Area-PHE	Area-LIG	Area-PHE
1	432502	422654	419316	417538
2	432659	433561	427219	426638
3	443723	428845	428103	419992
4	430101	436564	419719	427618
5	443852	425987	425138	423730
6	444955	422546	419944	427948
STD DEV	6877.673	5772.06	4043.327	4323.938
RSD	1.57	1.347	0.9553	1.02

Table 3
Within-day and week-to-week variation study of LIG and PHE[a]

	LIG individual Mean ± S.D.	LIG combination Mean ± S.D.	PHE individual Mean ± S.D.	PHE combination Mean ± S.D
Within-day	27.52 ± 0.403	27.45 ± 0.420	60.61 ± 0.510	27.45 ± 0.420
Week-to week				
I	27.43 ± 0.423	27.50 ± 0.417	60.54 ± 0.509	27.50 ± 0.417
II	27.50 ± 0.401	27.55 ± 0.410	60.67 ± 0.521	27.55 ± 0.410
III	27.52 ± 0.410	27.40 ± 0.405	60.50 ± 0.564	27.40 ± 0.405
IV	27.49 ± 0.401	27.50 ± 0.420	60.43 ± 0.510	27.50 ± 0.420

[a] $n = 3$.

3.4. Ruggedness studies

The results of the within-day and week-to-week variation evaluated by comparing the slopes of the standard curves of LIG and PHE prepared on the same and different days are depicted in Table 3. Low percentage variation between the values indicated excellent within day and between day reproducibility of the method (ANOVA; $P > 0.05$).

3.5. Recovery studies

The study was undertaken to document the efficiency of the extraction of the drugs from the DDS. The results of recovery of LIG and PHE from the DDS at low and high concentrations are presented in the Table 4. A recovery of greater than 98.99% for both the drugs indicated suitability of the extraction method.

3.6. Stability of LIG and PHE in solution

Determination of LIG and PHE from the solution at various time intervals up to 120 min did not show any degradation, indicating stability of these drugs in the solution during analysis (analysis time ≈ 60 min).

Table 4
Recovery studies[a]

Drug	Conc (μg)	Recovery (%)
LIG	12	98.99 ± 0.45
	16	99.06 ± 1.04
PHE	5	99.73 ± 0.59
	8	100.43 ± 1.42

[a] $n = 3$.

Table 5
Analysis of DDS[a]

Drug	Actual conc mg/DDS	Expt. conc mg/DDS	Difference (%)	RSD (%)
LIG	20	19.97 ± 1.05	0.15	5.25
PHE	0.5	0.489 ± 0.02	2.24	4.08

[a] $n = 3$.

3.7. Analysis of DDS

Based on the concentration of LIG and PHE in the DDS, two separate aliquots were spotted namely 3 μl for LIG and 50 μl for PHE to obtain drug concentrations in the linear range. Results of analysis of DDS containing LIG and PHE given in Table 5 indicate that the present method can be successfully used to estimate LIG and PHE when present in combination in pharmaceuticals.

4. Conclusions

LIG and PHE show maximum UV absorption at 262 and 273 nm. Hence, an appropriate method of estimation when these drugs are administered together is chromatographic analysis. HPTLC determination of LIG and PHE from DDS revealed no interference between two drugs and from the polymeric excipients of the DDS and also the method is rapid, allowing a high sample throughput necessary for routine analysis with an added advantage of low solvent consumption. The method described herein is simple, rapid, selective method and well suited for quantitative estimation of LIG and PHE individually and in combination both as bulk drug and from pharmaceutical preparations.

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