ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 1229–1232

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Comparision of high-performance and thin-layer chromatographic methods for the assay of lidocaine[1]

Ljiljana Zivanovic[a], S. Agatonovic-Kustrin[a], M. Vasiljevic[a], I. Nemcova[b]

[a]*Institute of Pharmaceutical Chemistry and Drug Analysis, Faculty of Pharmacy, Vojvode Stepe 450, 11 000 Belgrade, Yugoslavia*
[b]*Charles University, Prague, Czech Republic*

Received for review 13 September 1995; revised manuscript received 14 November 1995

Abstract

Reversed-phase high-performance liquid chromatographic (HPLC) and thin-layer chromatographic (TLC)–UV densitometric methods were developed for the quality control of lidocaine hydrochloride bulk drug and its injection solutions. The HPLC method used an RP-18 reversed-phase column with methanol–water–1% phosphoric acid–hexylamine (30:70:100:1.4, v/v/v/v) as the mobile phase and detection at 254 nm, with a capacity factor $k' = 0.8$. The TLC–UV densitometric method was performed on silica gel plates using diisopropyl ether–acetone–diethylamine (85:10:5, v/v/v) as the developing solvent and UV detection at 254 nm. The response was linear up to 10 $\mu g\ ml^{-1}$ (HPLC) and 8 $mg\ ml^{-1}$ (TLC). The RSD of the peak areas was 1.71% for HPLC and 0.55% for TLC, with recoveries in the range 99.6–100.2% for HPLC and 99.2–100.7% for TLC.

Keywords: Lidocaine hydrochloride bulk drug and injections; Reverse-phase high-performance liquid chromatography; Thin-layer chromatography–UV densitometry

1. Introduction

Lidocaine (2-diethylaminoaceto-2′,6′-xylilide) is a potent local anaesthetic. Methods for the determination of lidocaine hydrochloride in pharmaceutical preparations are generally based on spectrophotometry [1–4], high-performance thin-layer chromatography (TLC) [5,6] and TLC–densitometry [7]. Gas–liquid chromatography [8] and HPLC [9,10], have been extensively applied to the determination of lidocaine hydrochloride in biological fluids in recent years. Accordingly, the aim of this work was to develop an HPLC method for the determination of lidocaine hydrochloride in liquid dosage forms and to compare the results obtained by reversed-phase (RP)-HPLC and TLC–UV densitometry.

[1] Presented at the Fifth International Symposium on Drug Analysis, September 1995, Leuven, Belgium.

SSDI 0731-7085(95)01701-1

Table 1
Precision of assay for lidocaine hydrochloride

Parameter	RP-HPLC			TLC–UV densitometry		
	Lidocaine hydrochloride bulk drug (ng per 10 μl)[a]			Lidocaine hydrochloride bulk drug (μg per 10 μl)[a]		
Taken	40.00	50.00	60.00	30.00	40.00	50.00
Found	39.70	49.90	60.30	29.90	40.10	49.70
SD	0.75	0.85	1.03	0.18	0.22	0.36
RSD (%)	1.88	1.71	1.72	0.60	0.55	0.72
Relative error (%)	0.75	0.40	0.50	0.33	0.25	0.60

[a] $n = 20$.

2. Experimental

2.1. Materials

Lidocaine hydrochloride, methanol, phosphoric acid, hexylamine, acetone, diisopropyl ether, diethylamine and silica gel GF_{254} plates were obtained from Merck (Darmastadt, Germany). Lidokain hlorid injections at 20 mg per 2 ml and 40 mg per 2 ml were obtained from ICN Galenika (Belgrade, Yugoslavia).

2.2. Equipment

UV densitometric analyses were performed with an HPLC scanner from Camag (Muttenz, Switzerland). The solutions were applied with a Camag Linomat III. HPLC analysis were performed with a Perkin-Elmer Series 3B liquid chromatograph (Norwalk, CT) with a Perkin-Elmer UV detector and a Rheodyne model 7125 injector valve (10 μl sample loop) and a Hypersil C_{18} 5 μm columns (15 cm × 4.6 mm i.d.).

2.3. Solutions

Standard solutions of lidocaine hydrochloride for TLC analysis were prepared in absolute ethanol and the calibration graph was prepared with eight solutions having concentrations of 10–80 μg ml^{-1} (3.7×10^{-3}–2.9×10^{-2} mol l^{-1}). A freshly prepared 40 μg per 10 μl ethanol solution of lidocaine hydrochloride bulk drug was used as a stock solution for TLC analysis. Aqueous solutions of lidocaine hydrochloride from Lidokain hlorid ampoules was diluted with absolute ethanol to a concentration of 40 μg per 10 μl.

Standard solutions of lidocaine hydrochloride for HPLC were prepared in mobile phase and the calibration graph was obtained using ten standard solutions with concentrations ranging from 10 to 100 ng/10 μl (3.6×10^{-6}–3.6×10^{-5} mol l^{-1}). The concentration of lidocaine hydrochloride stock solutions was 50 ng per 10 μl. Aqueous solutions from Lidokain hlorid ampoules were diluted with mobile phase to a lidocaine hydrochloride concentration of 50 ng per 10 μl (1.8×10^{-5} mol l^{-1}).

2.4. Chromatographic procedure

For the TLC determination of lidocaine hydrochloride, chromatographic plates (20 × 20 cm) coated with silica gel GF_{254}, layer thickness 0.25 mm, were used. Volume of 10 μl of each solution were applied on the starting line of the plate. The mobile phase was diisopropyl ether–acetone–diethylamine (85:10:5, v/v/v). The migration zones of lidocaine hydrochloride were detected under UV light at 254 nm. The measurements of the zone areas were performed with an HPTL scanner at 254 nm, reading being directly from the thin layer. The lidocaine hydrochloride content was calculated according to the equation $K_a = P_a K_{STD}/P_{STD}$, where K_a is the amount of active substance, K_{STD} is amount of standard substance and P_a and P_{STD} are the peak areas of active substance and standard, respectively.

HPLC analysis was performed with an injection valve with a 10 μl sample loop and UV detection at 254 nm. The mobile phase was methanol–water–1% phosphoric acid–hexylamine (30:70:100:1.4, v/v/v/v). A reversed-phase column was employed at 25°C with a flow rate of 1 ml min^{-1}. The sensitivity was kept at 0.2 a.u.f.s. Injections of 10 μl were used for all solutions to be analysed. The lidocaine solutions were injected directly into the chromatograph.

3. Results and discussion

The working conditions for the RP-HPLC and TLC–UV densitometric methods were established with lidocaine hydrochloride bulk drug and then applied to the liquid dosage forms. HPLC was performed on a Hypersil C_{18}, 5 μm column. Methanol–water–1% phosphoric acid–hexylamine (30:70:10:1.4, v/v/v/v) mixture was found to be a good solvent both for elution and for dilution of lidocaine hydrochloride in its injections. An aliquot of the solution of lidocaine hydrochloride was injected into the HPLC system at a flow rate of 1 ml min^{-1} and the effluent was monitored with a UV detector at 254 nm. The retention time of lidocaine hydrochloride was 4.5 min.

TLC–UV densitometry [7] was performed on silica gel GF_{254} plates. Nine samples were spotted at the start point. The chromatogram was developed with diisopropyl ether–acetone–diethylamine (85:10:5, v/v/v). Optimum conditions for measurments with the HPLC scanner were established experimentally: wavelength 254 nm, slit width 2 mm (micro position), speed 1 mm s^{-1}, mode selector linear measure and scanning parameters $A = 1$ cm, $B = 2$ cm.

The Beer's law plots were found to be linear over the concentration rages 10–100 ng per 10 μl for HPLC and 10–80 μg per 10 μl for TLC. Over these concentration ranges, linear regression analysis of the lidocaine hydrochloride peak area (y) versus lidocaine hydrochloride concentration (x) ($n = 10$) yielded the equations $y = 0.1201 + 0.06323x$ ($r = 0.9999$) for HPLC and $y = 0.4936x - 0.0836$ ($r = 0.9997$) for TLC.

The precision of the methods was determined with three different concentrations of lidocaine hydrochloride, as shown in Table 1. The RSD of the peak areas was 1.71–1.88% for HPLC and 0.55–0.72% for TLC. The applicability of the methods for the assay of sample dosage forms was determined by dermining lidocaine hydrochloride in Lidokain hlorid injections ($n = 20$). Low values of SD (0.24–0.26 ng per 10 μl for TLC and 0.87–0.91 ng per 10 μl for HPLC) show the accuracy and reproducibility of the methods (Table 2). The high recoveries of lidocaine hydrochloride injection solution of 99.6–100.20% for HPLC and 99.25%–100.7% for TLC (Table 2) indicate that both methods are suitable for the determination lidocaine hydrochloride bulk drug and injections.

The HPLC method provides nanogram sensitivity and adequate linearity and repeatibility, but lower accuracy and precision in comparison with TLC–UV densitometric method, but the differences are not significant, as shown in Table 3. The proposed methods are rapid and simple, which is important for routine application.

Table 2
Recovery of lidocaine hydrochloride from pharmaceutical preparations

Parameter	RP-HPLC		TLC–UV densitometry	
	Concentration (ng per 10 μl)		Concentration (μg per 10 μl)	
	A[a]	B[b]	A[a]	B[b]
Taken	50.00	50.00	40.00	40.00
Found (x)	49.80	50.10	39.70	40.30
X_{min}	49.60	49.90	39.50	39.90
X_{max}	49.90	50.20	39.90	40.40
SD	0.91	0.87	0.26	0.24
RSD (%)	1.82	1.84	0.65	0.60
Recovery (%)	99.60	100.20	99.25	100.70

[a] Sample: Lidokain hlorid injections at 10 mg ml^{-1} ($n = 20$).
[b] Sample: Lidokain hlorid injections at 20 mg ml^{-1} ($n = 20$).

Table 3
Comparison of RP-HPLC and TLC–UV densitometric methods for lidocaine hydrochloride assay

Parameter	RP-HPLC		TLC–UV densitometry	
	Lidocaine hydrochloride[a]		Lidocaine hydrochloride[a]	
	Bulk drug	Injections	Bulk drug	Injections
SD	0.75–1.03[b]	0.87–0.91	0.18–0.36[c]	0.24–0.26[a]
RSD (%)	1.71–1.88	1.74–1.82	0.55–0.72	0.60–0.65
Relative error (%)	0.40–0.75		0.25–0.60	
Recovery (%)		99.60–100.20		99.25–100.70

[a] $n = 20$.
[b] ng per 10 μl.
[c] μg per 10 μl.

References

[1] M. Syoyama and T. Sano, Yagugaki Zasshi, 104 (1984) 351–355.
[2] J. Lemli and J. Knockaert, Pharm. Weekbl., Sci. Ed., 5 (1983) 142–144.
[3] G. Ezzat and M. Soad, Egypt. Pharm. Sci. 18 (1980) 355–366.
[4] M. Fayez, M.El-Tarras and S. Zeinab, Chem. Biomed. Environ. Instrum., 11 (1981) 411–423.
[5] T.F. Noggle and R.C. Clare, J. Assoc. Off. Anal. Chem., 66 (1983) 151–157.
[6] J.L. Duschi and P.L. Hackett, J. Anal. Toxicol., 9 (1985) 67–70.
[7] Lj. Zivanovic, D. Zivanov-Stakic and D. Radulovic, J. Pharm. Biomed. Anal., 6–8 (1988) 809–812.
[8] Y. Chen, J.M. Potter and P.J. Ravenscroft, J. Chromatogr., 574 (1992) 361–364.
[9] Y. Chen, J.M. Potter and P.J. Ravenscroft, J. Chromatogr., 574 (1992) 361–364.
[10] J. Klein, D. Fernandez, M. Gazarian, G. Kent and G. Koren, J. Chromatogr., Biomed. Appl., 651 (1994) 83–88.