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Journal of Pharmaceutical and Biomedical Analysis 47 (2008) 190–194
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

HPLC detection of marker compounds during buccal permeation enhancement studies

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Received 16 October 2007; received in revised form 27 November 2007; accepted 6 December 2007
Available online 23 December 2007

Abstract

A simple, isocratic and sensitive high-performance liquid chromatographic (HPLC) method was developed and validated for the simultaneous analysis of marker compounds for the aqueous (atenolol) and lipoidal (lidocaine) pathways during permeation enhancement studies across the buccal mucosa. A reversed-phase C_{18} column with UV detection at 224 nm was used for chromatographic separation and analysis, respectively. The mobile phase contained a mixture of acetonitrile–methanol–monobasic potassium phosphate (pH 3.0; 50 mM) (7.5:7.5:85, v/v/v). The permeabilities of marker compounds were determined across porcine buccal mucosa, which was either untreated (control) or pre-treated with sodium glycodeoxycholate (GDC-Na; 10 mM). The calibration curve showed good linearity over the concentration range of 0.1–25 μg/mL. The intra- and inter-day accuracy and precision were also within acceptable limits. The application of this method was demonstrated by an increase in the permeation of atenolol after pre-treatment with GDC-Na while the permeation of lidocaine did not change significantly.

Keywords: Buccal; Flux; Permeation enhancement; Bile salt; Reversed-phase chromatography

1. Introduction

Buccal mucosa is an attractive route for drug delivery due to its rich blood supply, good accessibility for administering drugs and lack of first-pass metabolism [1,2]. Based on the biochemical composition and structure of the buccal mucosa, drugs can permeate across the buccal mucosa by the lipoidal and/or aqueous pathways [3–6]. One major disadvantage with drug delivery across the buccal mucosa is poor permeability relative to the intestinal epithelium, which might result in sub-therapeutic concentrations for some drugs [7]. One approach to overcome this limitation is the incorporation of permeation enhancers in the buccal drug delivery system. Various permeation enhancers such as surfactants, bile salts, fatty acids, ethanol, and azone act by increasing the permeability of drugs through the lipoidal and/or aqueous pathways [7]. However, toxic effects like irreversible damage to the tissue have hampered the use of these enhancers. An understanding of the barrier properties and the permeation enhancement mechanisms may help in screening new enhancers [8].

At present, no single method is reported for the simultaneous analysis of marker compounds for permeation across the two pathways in the buccal mucosa. Therefore, the objective of this study was to develop a simple, isocratic and sensitive high-performance liquid chromatographic (HPLC)-UV method for the analysis of model marker compounds, which can be used for screening permeation enhancers in the buccal mucosa. Atenolol and lidocaine were used as marker compounds (Table 1) for the aqueous and lipoidal pathways, respectively, in the buccal mucosa. The applicability of the assay was demonstrated by permeation studies across the buccal mucosa using 10 mM sodium glycodeoxycholate (GDC-Na) as the permeation enhancer.

2. Materials and methods

2.1. Materials

Atenolol, lidocaine hydrochloride, and GDC-Na were purchased from Sigma (St. Louis, MO). HPLC grade solvents were purchased from Fisher Scientific (New Jersey). All other

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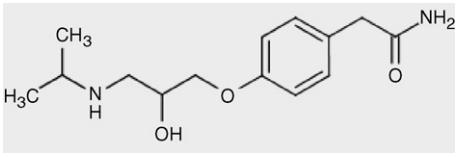
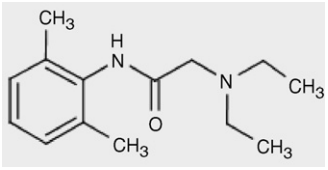
doi:10.1016/j.jpba.2007.12.020

Table 1
Structures and physicochemical properties of atenolol and lidocaine

Drug	Atenolol	Lidocaine
Structure		
Molecular weight	266	234
log P[a]	0.16	2.44
log $D_{6.8}$[b]	−1.3	1.2
pK_a[c]	9.54	7.9

[a] The partition coefficient (log P) values were obtained from ChemIDplus Advanced (National Library of Medicine).
[b] The values for distribution coefficient at pH 6.8 (log $D_{6.8}$) were obtained from Refs. [17,18].
[c] pK_a values were obtained from Refs. [19,20].

reagents were of analytical grade and used as received. Deionized water was used in the preparation of transport medium (buffer) and mobile phase.

2.2. Apparatus and chromatographic conditions

The apparatus used for the HPLC analysis was a Waters system (MA, USA) equipped with a Waters 510 pump, Waters 717 plus autosampler, and a Shimadzu SPD-10A UV detector (Kyoto, Japan). An ODS-AQ C_{18} column (4.6 mm × 150 mm; 5 μm particle size; YMC Brand, Waters, MA) was employed during the analysis. An isocratic method was used with a mobile phase containing a mixture of acetonitrile, methanol and monobasic potassium phosphate (pH 3.0; 50 mM) (7.5:7.5:85, v/v/v) at a flow rate of 1.0 mL/min. The pH of the buffer was adjusted to 3.0 with concentrated phosphoric acid followed by the addition of acetonitrile and methanol. As contaminants eluted out of the buccal tissue in a time range of 1–3 min, the mobile phase was selected to give a retention time of greater than 4 min for both the compounds. The column was maintained at room temperature (25 ± 2 °C). The wavelength and injection volume were set at 224 nm and 50 μL, respectively.

2.3. Preparation of calibration standards

A primary stock solution of atenolol and lidocaine with a concentration of 1.0 mg/mL was prepared in a pH 6.8 phosphate buffer. A secondary stock solution (200 μg/mL) was prepared by dilution of the primary stock solution with drug-free buffer, equilibrated with the buccal mucosa (matrix). The matrix was prepared by incubating the buffer with buccal tissues in side-by-side diffusion cells for 11 h at 37 °C. Aliquots of the secondary stock were further diluted with the matrix to obtain six calibration standards (0.1, 0.5, 1.0, 5.0, 10.0, and 25.0 μg/mL). The mobile phase and calibration standards were freshly prepared on the day of use.

2.4. Method validation

The chromatographic methods for atenolol and lidocaine were validated by determining parameters such as linearity, accuracy, precision, selectivity, and sensitivity [9]. The linearity of the calibration curves was obtained by injecting the six calibration standards ($n = 5$ for each concentration). The accuracy and precision were evaluated by analysis of the six calibration standards and three different quality control (QC) samples (0.3, 4.0, and 15.0 μg/mL). The QC samples are defined as analyte samples of known concentrations representing the entire range of the calibration curve [9]. The accuracy is defined as the percentage relative error (%R.E.), which is measured as the deviation between the measured concentration and the nominal concentration value: [(measured value − nominal value)/nominal value] × 100 [10]. Intra-day accuracy was calculated by injecting replicates ($n = 5$) of each concentration. Precision (%R.S.D.) is defined as the percentage of the standard deviation divided by the mean value for each concentration [10]. Intra- and inter-day precisions were calculated by analyzing calibration standards and QC samples on three consecutive days ($n = 5$ on day 1 and $n = 3$ on days 2 and 3) [11].

The selectivity of the assay was verified by analyzing blank matrix samples for interfering peaks [12]. The sensitivity of the method was determined by measuring the limit of quantitation (LOQ), which is defined as the lowest standard concentration at which the accuracy and precision are lower than 15% [13]. The limit of detection (LOD) is defined as one-third concentration of LOQ [11].

2.5. Tissue preparation

Porcine buccal tissue was obtained from a local ranch immediately after the pigs were slaughtered. The tissues were stored in phosphate buffer, pH 6.8 during transport and processing. Buccal epithelium was separated from the underlying connective tissue by trimming the latter to a thickness of 500 ± 50 μm with a surgical scissor. The permeation studies were initiated within 2 h of slaughtering.

2.6. Permeation studies

In vitro permeation studies were conducted at 37 °C using horizontal, water-jacketed, side-by-side cells (PermeGear Inc.,

Riegelsville, PA) with a diffusion area of 0.68 cm^2. The tissue was mounted between the donor and receiver chambers followed by equilibration with a phosphate buffer (pH 6.8) for 30 min. The donor chamber was charged with a mixture of atenolol (20 mg/mL) and lidocaine HCl (1 mg/mL) dissolved in the buffer. Samples (1.0 mL) were withdrawn from the receiver every 30 min for the first 3 h and at 2.0 h intervals after that over a period of 11 h. The receiver chamber was replenished with fresh buffer after every sampling point. For enhancement studies, the tissues were treated with GDC-Na (10 mM) in the donor chamber for 1.5 h followed by 3 × 10 min rinsing with fresh buffer. The drug mixture was then added to the donor chamber and aliquots of receiver fluid were sampled as mentioned above.

The apparent permeability coefficient, P_{app} (cm/s) was calculated from the permeation studies using the following equation:

$$P = \frac{J_{ss}}{C \times 3600} = \frac{\Delta Q/\Delta t}{A \times C \times 3600} \quad (1)$$

where J_{ss} is the steady-state flux (μg/h/cm^2), $\Delta Q/\Delta t$ is the steady-state rate of appearance of the drug in the receiver chamber (μg/h), A is the diffusional area (cm^2), and C is the initial drug donor concentration (μg/mL).

The permeation enhancement ratios (ER) after pre-treatment with GDC-Na were calculated according to Eq. (2)

$$ER = \frac{P_{(enh)}}{P_{(ctrl)}} \quad (2)$$

where $P_{(enh)}$ is the apparent permeability coefficient of the marker compound after pre-treatment with GDC-Na and $P_{(ctrl)}$ is the apparent permeability coefficient across untreated (control) buccal mucosa.

Studies were carried out in triplicate and the results were expressed as mean ± S.D. The statistical difference between drug permeabilities across GDC-Na untreated and pre-treated buccal mucosa was evaluated by the Student's *t*-test.

3. Results and discussion

3.1. Chromatography

A Zorbax SB-C_{18} column (4.6 mm × 150 mm) (Agilent Technologies, Santa Clara, CA) was initially used for separation of the marker compounds. However, the atenolol peak (retention time = 2.1 min) coincided with the peaks due to the endogenous compounds eluting from the buccal mucosa during the permeation studies, thereby resulting in significant inter-

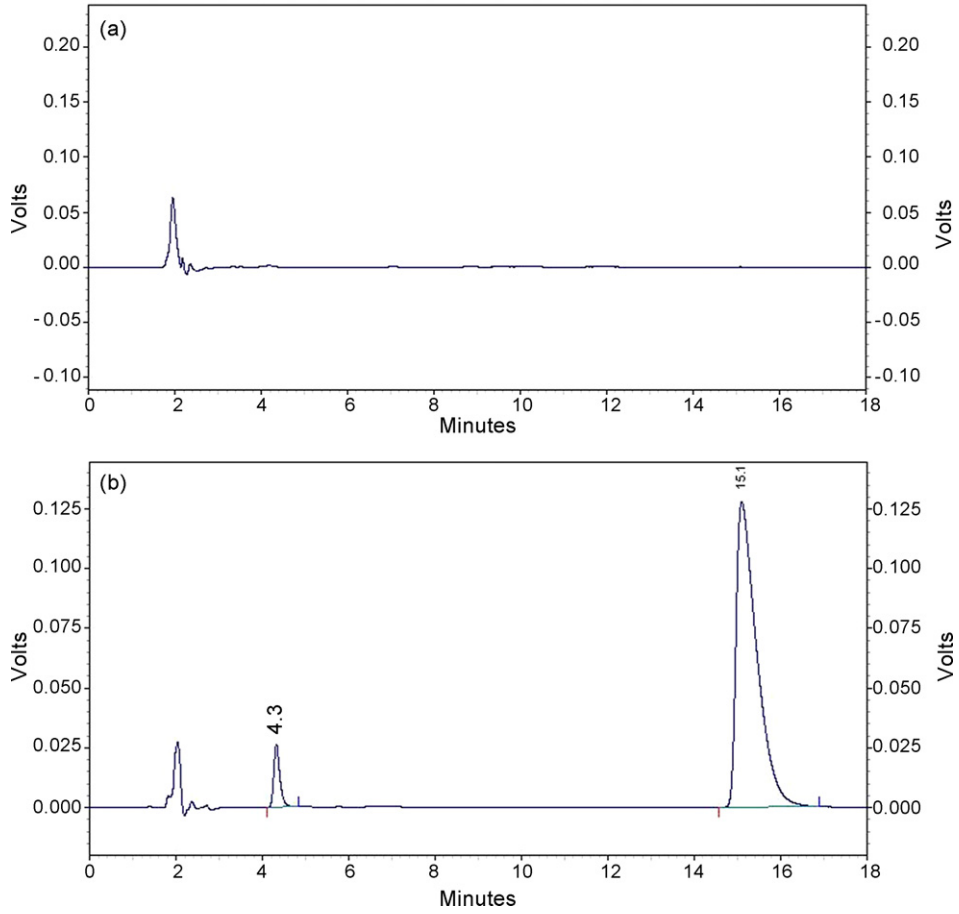


Fig. 1. Chromatograms of (a) blank buccal buffer matrix and (b) permeation sample withdrawn at 11.0 h. The retention times of atenolol and lidocaine were 4.3 and 15.1 min, respectively.

ference. Therefore, a YMC-Pack ODS-AQ column was used subsequently due to the hydrophilic nature of the endcapping, which resulted in a stronger retention of polar sample solutes [14]. The composition of the mobile phase was adjusted to provide the best peak resolution and retention times. In addition, an isocratic method is attractive over the gradient due to its simplicity. At an optimum wavelength of 224 nm, both analytes (atenolol and lidocaine) had a good response. Under the chromatographic conditions used, atenolol and lidocaine had retention times of 4.3 and 15.1 min, respectively. Representative chromatograms of blank matrix and a permeation sample are shown in Fig. 1. The method was selective for the marker compounds as there was no interference from the endogenous compounds of the buccal mucosa. Although there was some tailing in case of lidocaine, the tailing factor (T_f) of 1.5 observed in this study was within permissible limits (recommended $T_f \leq 2.0$) [15].

3.2. Method validation

3.2.1. Linearity and sensitivity

Excellent linearity was obtained in the concentration range of 0.1–25.0 μg/mL for both analytes. The correlation coefficients were greater than 0.999 for all the six point standard curves obtained on different days. The equations for the calibration lines were as follows: $y = 161288.2x + 4139.4$ for atenolol and $y = 76887.8x - 3657.4$ for lidocaine, where x is the drug concentration (μg/mL) and y is the peak area. The standard errors for the slope and intercept were 129 and 2447, respectively for atenolol and 358 and 4005, respectively for lidocaine. The slope was significantly different from 0 and the intercept was not significantly different from 0 ($p < 0.05$). The LOQ and LOD were 100.0 and 33.3 ng/mL, respectively for both atenolol and lidocaine.

3.2.2. Accuracy and precision

The calibration and QC standards were analyzed on three consecutive days to assess the accuracy (intra-day; %R.E.) and precision (intra- and inter-day; %R.S.D.). The results are summarized in Table 2. The precision and accuracy values for the QC samples were less than 3.5%. In addition, the precision and accuracy values for the calibration standards were within acceptable limits (<10%). According to U.S. Food and Drug Administration (FDA) guidelines, the accuracy should be within ±15%, except at LOQ, where it should not exceed ±20%. Similarly, the precision around the mean value should not exceed 15%, except at LOQ, where it should be less than 20% [9,16]. Therefore, the values obtained in this study are within permissible limits.

Individual calibration solutions of atenolol and lidocaine with a concentration of 25.0 μg/mL were prepared and analyzed to investigate the presence of interactions between the two compounds when dissolved together. The %R.E. values (as separate solutions and as a mixture) for atenolol and lidocaine were determined to be 1.56 and 1.84, respectively. This suggested that the analysis of these two marker compounds in combination showed no interaction.

Table 2
Accuracy and precision of the calibration and QC standards of atenolol and lidocaine

Marker concentration (μg/mL)	Atenolol			Lidocaine		
	Intra-day[a]		Inter-day[b]	Intra-day[a]		Inter-day[b]
	%R.S.D.	%R.E.	%R.S.D.	%R.S.D.	%R.E.	%R.S.D.
Calibration standards						
0.1	6.48	3.94	5.86	7.79	1.61	9.89
0.5	2.44	7.05	2.67	3.32	4.46	3.00
1.0	0.22	2.61	0.90	2.42	−0.95	3.19
5.0	0.78	0.46	1.31	1.33	−2.77	1.69
10.0	0.60	0.49	0.65	0.49	−1.83	0.59
25.0	0.37	0.07	0.56	0.31	0.09	0.46
Quality control samples						
0.3	1.50	1.00	1.64	3.41	−2.42	3.33
4.0	0.42	0.59	0.69	1.20	−2.80	1.01
15.0	0.70	2.46	1.03	0.54	1.00	1.16

[a] Intra-day accuracy and precision were determined using $n = 5$ on day 1 for each calibration and QC standard.
[b] Inter-day precision was determined using $n = 11$ (day 1: $n = 5$; days 2 and 3: $n = 3$) for each calibration and QC standard.

3.3. Application of the chromatographic method

The applicability of the assay was demonstrated by permeation enhancement studies across the buccal mucosa. Atenolol and lidocaine were selected as model compounds for the aqueous and lipoidal pathways, respectively, on the basis of their lipophilicities ($\log D_{6.8}$) [17–20]. Although lidocaine was used as a hydrochloride salt, a $\log D_{6.8}$ of 1.2 indicates a greater affinity for the lipoidal pathway than the aqueous pathway. However, it should be noted that in addition to permeant lipophilicity, the P_{app} of ionizable molecules depends on the pH conditions and drug pK_a. The concentration of GDC-Na used in this study (10 mM) was greater than the reported CMC of 4 mM [21]. The P_{app} of atenolol was observed to be $(2.7 \pm 1.1) \times 10^{-8}$ cm/s in case of the untreated (control) buccal mucosa. However, after pre-treatment with GDC-Na, the P_{app} increased to $(23.9 \pm 5.6) \times 10^{-8}$ cm/s, i.e., a 9-fold (ER = 9) increase was observed (Fig. 2). However, there was no significant change ($p > 0.05$) in the permeability of lidocaine (ER = 1) after pre-treatment with the permeation enhancer ($(17.1 \pm 1.6) \times 10^{-6}$ cm/s in case of control tissue *vs.* $(15.9 \pm 3.5) \times 10^{-6}$ cm/s in case of pre-treated tissue). It has been suggested that the pre-treatment of buccal tissue with a bile salt at concentrations in the vicinity of CMC results in solubilization of the intercellular lipids. As a result, the integrity of the intercellular domain in the buccal epithelium is compromised [22]. This leads to an enhanced permeability of hydrophilic drugs, which permeate predominantly through the aqueous (paracellular) pathway. It is also possible that the GDC-Na remaining in the tissue after pre-treatment and rinsing might lead to increased lipoidal permeability of the hydrophilic atenolol by ion-pairing. The results observed in this study are in agreement with a previous study from our laboratory [21,23,24].

The buccal permeabilities of atenolol and lidocaine were also determined separately without GDC-Na pre-treatment to investi-

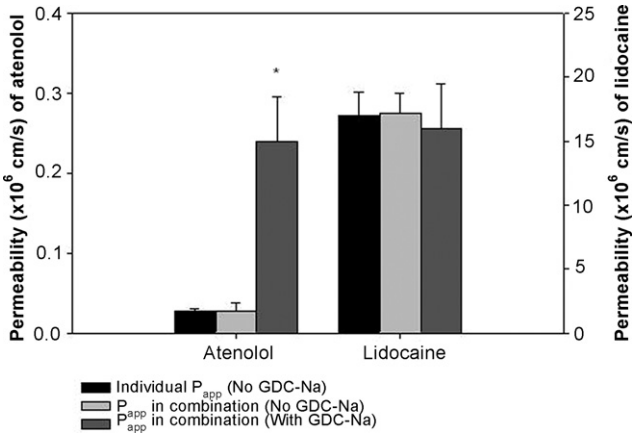


Fig. 2. Apparent permeability values across buccal mucosa (P_{app}) for atenolol and lidocaine (in combination) with and without GDC-Na (10 mM) pre-treatment. P_{app} was also measured individually for both the marker compounds without GDC-Na pre-treatment. *Significantly different ($p < 0.05$) P_{app} from the P_{app} in the absence of GDC-Na.

gate the presence of permeability altering effects of one marker on the other. The permeabilities of the marker compounds as individual entities and in combination were not significantly different ($p > 0.05$) (Fig. 2).

Several HPLC methods have been reported for the individual analysis of atenolol and lidocaine [25–29]. However, there is no evidence indicating that any of these methods could be used to analyze these two compounds simultaneously. To our knowledge, the current study reports the simultaneous analysis of these two compounds for the first time. The HPLC method developed and validated in this study can be implemented in the investigation of buccal permeation enhancement mechanisms of existing and novel enhancers.

4. Conclusion

A simple, isocratic HPLC method was developed and validated for the simultaneous analysis of atenolol and lidocaine, which were used as marker compounds during permeation enhancement studies in the buccal mucosa. The applicability of this method in buccal permeation enhancement studies was demonstrated using GDC-Na as a model enhancer.

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