Densitometric Determination of Mebhydrolin Napadisylate in Tablets*

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Key Words

Summary

A simple and rapid high-performance thin-layer chromatography (HPTLC) densitometric method has been developed for the determination of mebhydrolin napadisylate in tablets. After extraction of the analyte with a mixture of methanol and NH_4OH 25% (100:1.5, v/v), the extracts were spotted on precoated HPTLC silica gel F254 plates, which were developed with a mixture of methanol and ethyl acetate (1:1, v/v). Quantitative evaluation was performed by measuring the absorbance reflectance of the analyte spots at 287 nm. The method was validated for specificity, linearity, accuracy, precision, and robustness. Good linearity was achieved in the concentration range 600–1600 ng/spot. The RSD of repeatability and intermediate precision were found to be less than 2%, whereas the mean of the recovery data was 99.3–100.8%. The detection limit and quantification limit were 18.5 and 55.5 ng/spot, respectively. The drug was subjected to acidic and alkaline hydrolysis, oxidation, dry heat, and UV treatment. The peak of mebhydrolin napadisylate was not interfered by those of possible degradation products.

1 Introduction

Mebhydrolin napadisylate (CAS: 6153-33-9) is a sedating antihistamine with antimuscarinic and sedative properties. Usually, this drug is given orally for the symptomatic relief of allergic conditions such as urticaria, rhinitis, and pruritic skin disorders [1]. At present, many tablet preparations containing mebhydrolin napadisylate as the active ingredient are marketed in Indonesia [2]. Unfortunately, no official method for its analysis in the Indonesian Pharmacopeia [3] and other pharmacopeiae is available. *Sastry et al*. described a spectrophotometric method for the assay of mebhydrolin napadisylate and other antihistaminic drugs [4]. A high-performance liquid chromatography (HPLC) method for the determination of mebhydrolin napadisylate has been reported [5]. To the best of our knowledge, there is no publication reporting the determination of mebhydrolin napadisylate in pharmaceutical preparations using densitometry.

The objective of the present work is to develop a simple, rapid, and validated densitometry method for the determination of mebhydrolin napadisylate in tablets.

2 Experimental

2.1 Materials and Reagents

Mebhydrolin napadisylate (CrossChem, Lugano, Switzerland; Batch No. 20081001; Assay 99%, Expiry date: September 2013) was of pharmaceutical grade. Methanol, ethyl acetate, and NH_4OH 25% (E. Merck, Darmstadt, Germany) were of analytical grade reagents. The solvents were used without further purification.

Commercial tablets containing 76 mg mebhydrolin napadisylate were purchased from a local pharmacy in Jember, East Java, Indonesia (January 2010).

2.2 Standard Preparation

Stock standard solutions were always freshly prepared by dissolving accurately weighed mebhydrolin napadisylate (25 mg) in 25 mL solvent, which consisted of a mixture of methanol and NH_4OH 25% (100:1.5, v/v). Stock solutions were made twice by independent weighings. Various standard solutions were prepared from the two stock solutions by dilution with the solvent. For basic linearity studies, solutions were prepared containing 300, 400, 500, 600, and 800 µg mL^{-1}, and 2 µL of each of these solutions was spotted on the HPTLC plate. The standard solutions were stable at least for 4 h at room temperature in the solvent (mean ± RSD: 99.6% ± 2.8%) and on the HPTLC plates (mean ± RSD: 103.5% ± 1.5%).

A modified version of a published method was used for differentiating the spots of mebhydrolin base and napadisylate [6]. An amount of 20 mg mebhydrolin napadisylate was transferred into a 50-mL volumetric flask, and approximately 20 mL of solvent was added to it. The solution was then extracted four times by using 20 mL of chloroform in a vortex mixer. The chloroform
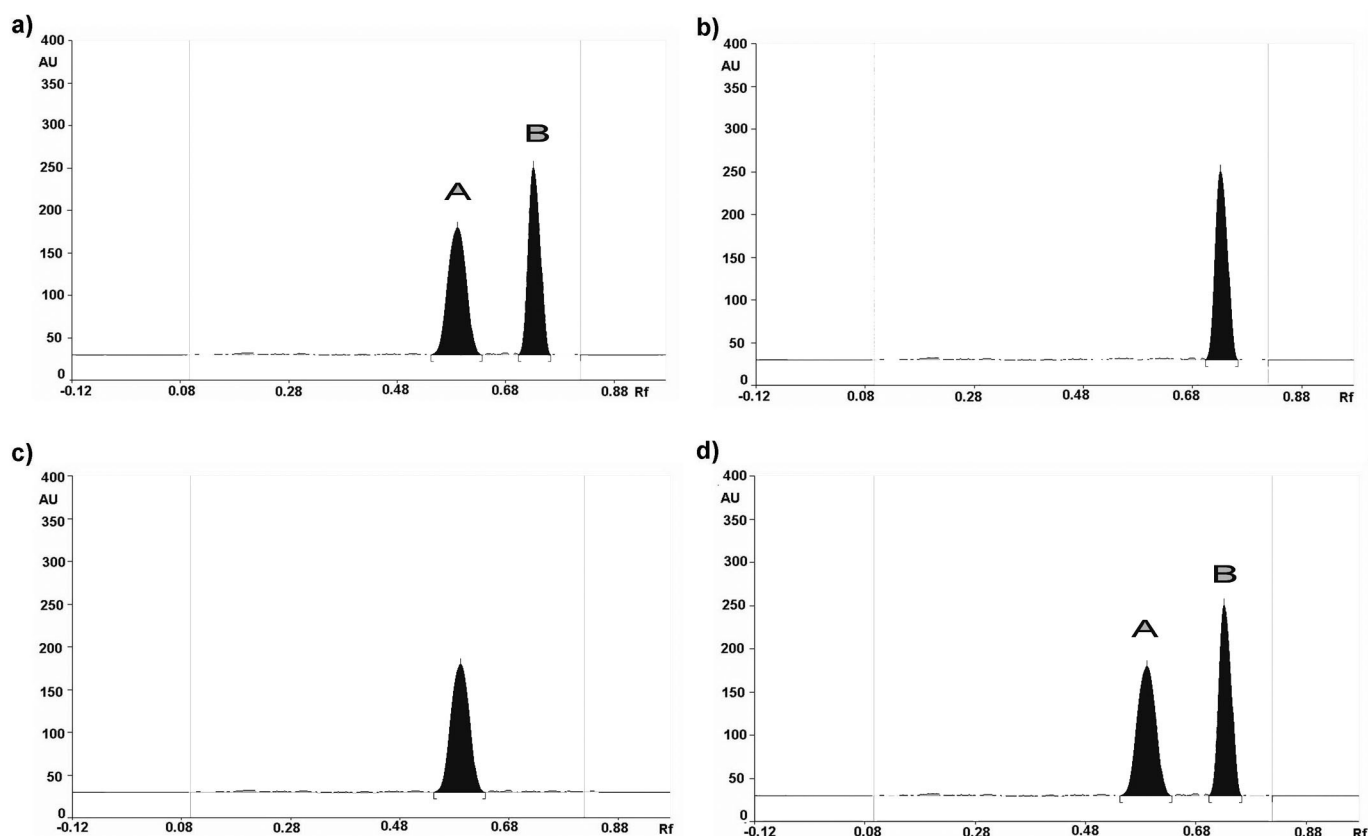
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Figure 1

Chromatograms (λ = 287 nm) obtained from standard solution of mebhydrolin napadisylate in methanol–ammonia (100:1.5, *v/v*) (a), F-Wt (b), F-Ch (c), and extract of mebhydrolin napadisylate in the commercial tablet (d).

fractions (F-Ch) were collected in a 25-mL vial. Two microliters of F-Ch and water fraction (F-Wt) were then spotted on the HPTLC plate.

2.3 Sample Preparation

Twenty tablets were weighed and their average weight was determined. The tablets were mixed and finely powdered. An amount equivalent to 12.5 mg of mebhydrolin napadisylate was transferred into a 25-mL volumetric flask, and approximately 15 mL of the solvent was added. The mixture was ultrasonicated for 5 min and then diluted to 25 mL with the same solvent. The solution was filtered through a Whatman type-40 filter paper. This solution (2 μL) was spotted on the HPTLC plates.

2.4 Chromatography

Chromatography was performed on precoated 20 cm × 10 cm HPTLC silica gel 60 F254 aluminum-backed sheets (E. Merck, #1.05548). A Nanomat III (CAMAG, Muttenz, Switzerland) equipped with 2.0 μL glass capillaries (CAMAG, Cat. 022.77772, CV $\approx$ 0.6%, $R \approx$ 0.25%) was used for sample application. The mobile phase used was a mixture of methanol and ethyl acetate (1:1, *v/v*). Ascending development was performed in a CAMAG HPTLC twin-trough chamber; the mobile phase migration distance in all experiments was 9 cm (development time was ca. 30 min at 25°C $\pm$ 2°C).

Densitometric scanning was performed with a CAMAG TLC Scanner III. The purity and identity of the analyte spots were

Table 1

Linear regression data.

Linearity range (n = 15)[a]	600–1600 ng/spot
Correlation coefficient (r)	0.996
Relative process standard deviation (V_{xo}) [7]	3.68%
Calculated sdv value (from CATS software)	3.62
Calculated F-value (ANOVA)	403.2 for $p <$ 0.0001
Calculated Fisher ratio of lack-of-fit error [8]	1.45 (<3.71; tabulated at the 95% with 3 and 10 degrees of freedom)
DL[b]	18.5 ng/spot
QL (3 × DL)	55.5 ng/spot

[a]Five levels of concentrations in triplicate (n = 5 × 3 = 15)

[b]DL = X_p (detailed calculation presented in text)

determined by scanning in the absorbance reflectance mode from 200 to 700 nm. Quantitative evaluation was performed by measuring the absorbance reflectance of the analyte spots at 287 nm. The densitometric scanning parameters were as follows: bandwidth 10 nm; slit 4 slit length 6; scanning speed 4 mm s^{-1}. Calculations for identity, purity checks ($r_{S,M}$ and $r_{M,E}$ where S = start, M = center; and E = end of spectrum), sdv (relative standard deviation of the linear curve), and quantifica-
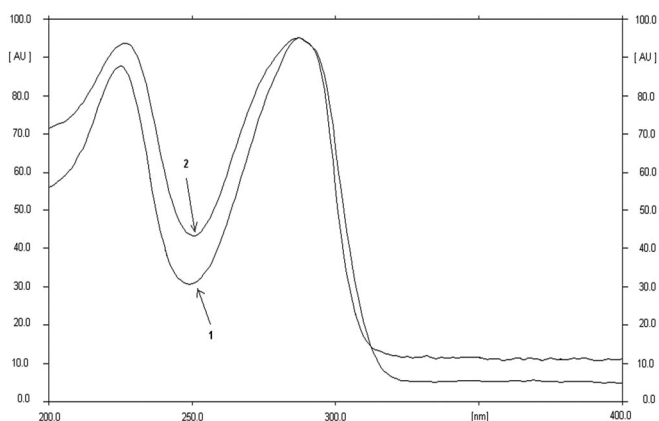
Figure 2

UV spectra of spot A of mebhydrolin napadisylate in the mixture of NH$_4$OH 25%–methanol (1.5:100, *v/v*) (1) and spot of Ft-Ch (2).

Figure 3

UV spectra of spot F-Wt (1), and spot B of mebhydrolin napadisylate in a mixture of NH$_4$OH 25%–methanol (1.5:100, *v/v*) (2).

tion of the analyte spots was performed by the CATS software version 1.4.1.8154 (2005) from CAMAG. Quantitative evaluations were performed via peak areas with linear regression, using at least four-point calibrations on each plate.

2.5 Validation Method

The method was validated for linearity, detection limit (DL), quantification limit (QL), accuracy, precision, and robustness according to the published methods [7–9] with appropriate modification. For accuracy studies, a five-point standard addition method on commercial tablets (addition 30%, 40%, 50%, 60%, and 70% of the label claim) was performed on three different days. The precision (repeatability, intermediate precision) and robustness were also evaluated by using commercial tablets.

2.6 Forced Degradation Studies

Approximately of 1000 mg (accurately weighed) of mebhydrolin napadisylate or powdered tablets (1000 mg) were mixed homogenously with 0.2 mL HCl 2N, NaOH 2N, and H$_2$O$_2$ 9%, and then incubated for 7 days at 60 °C. Dry heat degradation study was performed by storing the powdered samples at 80 °C for 7 days, while UV degradation study was carried out by irradiating the samples using a UV lamp at 254 nm (CAMAG) for 1 day. These stressed samples were then dissolved with the solvent as described in Section 2.2 and analyzed by the proposed densitometric method.

3 Results and Discussion

After the HPTLC plate was eluted, the densitogram at 287 nm of the standard solutions in the solvent and extract of commercial tablets showed two spots, namely, peak A (R_F ~0. 59) and peak B (R_F ~0.73) (**Figures 1a** and **d**). F-Ch (**Figure 1b**) and F-Wt (**Figure 1c**) yielded one spot, respectively (R_F 0.59 and 0.73). This indicated that the spots with R_F 0.59 and 0.73 were spots of the mebhydrolin base and napadisylate, respectively. This was confirmed by measuring their in situ UV spectra (**Figures 2** and **3**). Quantitative evaluation was performed on the peak of the mebhydrolin base only.
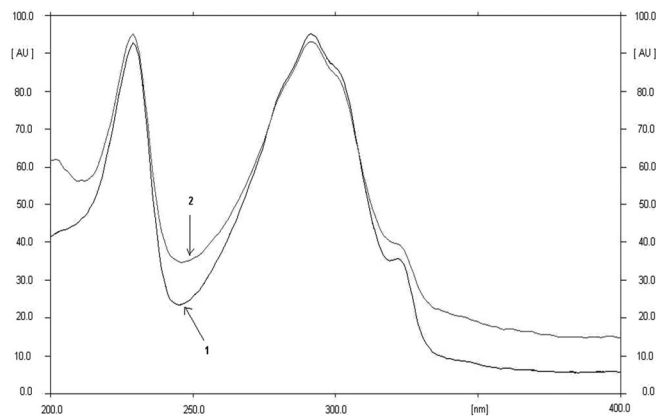
The proposed HPTLC system demonstrated that all analyte spots in samples furnished in situ UV spectra that were identical to those of standards ($r \geq 0.9999$). Purity check of the analyte spots using the CATS software also showed that all analyte spots of the extracts were pure. The values of $r_{S,M}$ and $r_{M,E}$ were ≥ 0.9999, demonstrating that the proposed HPTLC method is highly selective.

Linearity of mebhydrolin napadisylate was achieved from 600 to 1600 ng/spot obeying the equation $Y = 2.995X + 800.148$. The linear regression data is presented in **Table 1**. The basic calibration curve showed variable homogeneity over the whole range. The calculated test value of PW [7] was 4.09. The PW value was less than the F-table value (6.99; for $f_1 = 7, f_2 = 7; \alpha = 0.01$). The DL was determined by making a linear regression of relatively low concentrations of mebhydrolin napadisylate (20–200 ng/spot; $n = 9$; $V_{xo} = 4.03\%$; sdv = 4.21; $r = 0.9978$; line equation $Y = 5.58X + 11.35$). The ANOVA regression test showed a significant F-value (1621.3 for $p < 0.0001$). By this method, the calculated X_p value was 18.5 ng/spot; in this case DL $= X_p$ [7] (Table 1).

Table 2 demonstrates the high accuracy of the proposed method as revealed by the percentage of mean recovery data (99.3–100.8%). To test whether systematic errors had occurred, a linear regression of the recovery curve of X_f (percentage of label claim of the analyte found by the proposed method) against X_c (nominal percentage of label claim of the analyte after addition with the standard) of the commercial tablets was carried out. All recovery curve equations that are presented in **Table 3** did not reveal the occurrence of constant or proportional-systematic errors [7].

All the values of the repeatability and intermediate precision evaluations were less than 2% (**Table 4**). These values were also less than the required values (2.3%; specification range of 95–105%; $n = 6$) [10]. The three measurements were performed within one laboratory by same analyst on different plates and different days.

In order to evaluate the robustness of the proposed method, the influence of small variation on the composition of the mobile phase, volume of the mobile phase, and saturation time on the content of mebhydrolin napadisylate in the commercial tablets were evaluated (**Table 5**). The RSD values (%label claim) of the samples within treatment were less than 2%, and two-way

Table 2

Accuracy results of commercial tablets.

Pharmaceutical preparation	Ingredient	Label claim [%] Original[a] (mean ± %RSD)	Added [%]	Recovery (mean ± %RSD[b])
Commercial tablets	Mebhydrolin napadisylate	100.3 ± 1.22	30	100.7 ± 0.73
			40	99.3 ± 0.89
			50	100.8 ± 1.11
			60	100.5 ± 1.00
			70	100.1 ± 0.74

[a]$n = 18$

[b]Accuracy evaluations were performed in duplicate and three different days ($n = 2 \times 3 = 6$)

Table 3

The recovery curves of the accuracy studies.

Parameters	Recovery curve[a] Day 1	Day 2	Day 3
Mean of recovery [%]	99.8 ± 0.82	100.5 ± 0.82	100.3 ± 0.95
Recovery curve equation	$X_f = -0.04285 + 1.032X_c$	$X_f = -0.07879 + 1.068X_c$	$X_f = -0.00363 + 1.0058X_c$
V_{baf}[b]	−0.043 ± 0.169	−0.079 ± 0.160	−0.004 ± 0.200
V_{bbf}[c]	1.032 ± 0.135	1.068 ± 0.128	1.006 ± 0.160

[a]Experiment was performed on three different days

[b]Confidence range of intercept [7]

[c]Confidence range of slope [7]

Table 4

Results of precision evaluation.

Measurement[a]	RSD value [%] ($n = 6$)[b]
1	0.79
2	1.08
3	1.20

[a]Each measurement was performed by same analyst and on a different plate and different days.

[b]Evaluated by one analysts on one plate (repeatability)

Table 5

Robustness of the HPTLC method.

Parameter	% of label claim[a] (mean ± %RSD)	Two-way ANOVA p_1[b]	p_2[b]
(A) Mobile phase composition			
Methanol–ethyl acetate (9.5:10.5, v/v)			
Methanol–ethyl acetate (10.0:10.0, v/v)			
Methanol–ethyl acetate (10.5:9.5, v/v)	100.3 ± 1.41	0.742[A, B]	0.881[A, C]
(B) Mobile phase volume (18.0, 20.0, and 22.0 mL)	100.3 ± 0.96	0.742[B, A]	0.476[B, C]
(C) Duration of saturation (20, 30, and 40 min)	99.5 ± 1.58	0.881[C, A]	0.476[C, B]

[a]%RSD of label claim ($n = 6 \times 3 = 18$)

[b]Described the p-values to other factors (A, B, or C)

ANOVA evaluation showed that there was no significant difference between the treatments ($p > 0.05$). This shows that the proposed TLC method is robust.

The degradation tests showed that the content of the analytes as bulk substances and in tablets were reduced in the range of 0.2–8.2% under stressed conditions (**Table 6**). No degradation products were observed in the densitogram at UV 287 nm, which might be due the fact that the possible degradation products have no UV-absorbing functional group. The purity and identity check of the analyte spots of mebhydrolin napadisylate using the CATS software still yielded good values (>0.999), which showed that all the analyte spots were still pure and iden-

Table 6

Result of degradation studies.

Treatments	Content of mebhydrolin napadisylate[a] (mean ± %RSD; $n = 3$)	
	Bulk substance [%]	Tablets [% of label claim]
HCl 2N	92.5 ± 2.14	91.8 ± 3.6
NaOH 2N	98.3 ± 1.83	94.2 ± 0.71
H_2O_2 9%	98.3 ± 1.89	98.7 ± 2.22
Dry heat	97.3 ± 0.48	96.4 ± 2.15
UV 254 nm	99.8 ± 1.79	99.5 ± 0.81

[a]Purity and identity checks of analyte spots using CATS software yielded acceptable values ($r > 0.999$); no degradation product was observed (at 287 nm)

tical with the standard. This proved that the analyte peaks were not interfered by possible degradation products.

4 Conclusion

The present work showed that the proposed HPTLC densitometric method is simple, rapid, selective, precise, accurate, and robust. This method is suitable for the routine analysis of products of similar composition in the pharmaceutical industry quality control laboratories, especially for developing countries like Indonesia.

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