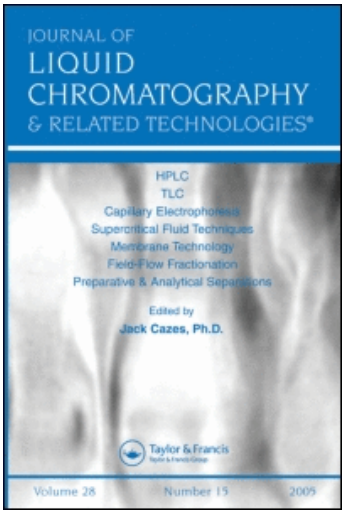


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# TLC Densitometric Determination of Bromhexine Hydrochloride in Pharmaceuticals, and Its Validation

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## ABSTRACT

A simple and rapid densitometric method has been developed for determination of bromhexine hydrochloride in pharmaceutical preparations. After dilution or extraction of the analyte using a mixture of acetone–water (2 : 1), the extracts were spotted on pre-coated silica gel plates, which were then developed with a mixture of *n*-butanol–glacial acetic acid–water (26 : 7.5 : 7.5). Quantitative evaluation was performed by measuring the absorbance–reflectance of the analyte spots at 325 nm. The densitometric method is selective, precise, and accurate and can be

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used for routine analysis of pharmaceutical preparations in pharmaceutical industry quality control laboratories.

*Key Words:* Bromhexine hydrochloride; Densitometric; Syrup; TLC; Tablet.



## INTRODUCTION

Many pharmaceutical preparations containing bromhexine hydrochloride [*N*-(2-amino-3,5-dibromobenzyl)-*N*-methylcyclo-hexanamine hydrochloride] as the active ingredient are marketed now in Indonesia.[1] Bromhexine hydrochloride is used for the treatment of respiratory disorders associated with productive cough, and can been used also for the treatment of dry eye in Sjögren's syndrome.[2] The official methods for its determination were using titration and spectrophotometric methods.[3–6]

Some spectrophotometric,[7–9] capillary electrophoresis,[10,11] gas chromatography,[12,13] flow injection,[14] high performance liquid chromatography,[15,16] and thin layer chromatography (TLC)[17] methods have been published for the determination of bromhexine hydrochloride in pharmaceutical preparations and biological fluids. Unfortunately, most of the methods using TLC is still using chloroform as one of the components of the mobile phase. Using chloroform in an industrial environment is hard to justify today due to its toxicity. It is well known that chloroform is a carcinogenic, mutagenic, and teratogenic substance.

The aim of this work was to develop a simple, safe, cheap, and rapid TLC densitometric method for routine analysis of bromhexine hydrochloride in pharmaceutical preparations.

## EXPERIMENTAL

### Materials and Reagents

Bromhexine hydrochloride (Transo-Pharm, Hamburg, Germany, Batch BR-11526, assay: 99.66%) was of pharmaceutical grade substance, and was used as received for preparing laboratory-made pharmaceutical preparations and standard solutions.

Acetone (E. Merck, Darmstadt, Germany), *n*-butanol, and glacial acetic acid (Mallinckrodt Baker Inc, Phillipsburg, NJ) were analytical grade reagents. The solvents were used without further purification. Excipients for laboratory-made syrup preparations (citric acid, sodium citrate, sodium chloride, sodium

saccharin, sodium cyclamate, nipagin, nipasol, propylene glycol, alcohol 96%, and sorbitol) were pharmaceutical grade substances.

Laboratory-made syrup preparations were prepared containing five different concentrations of bromhexine hydrochloride (38.4, 43.2, 48.0, 52.8, and 57.6 mg 60 $mL^{-1}$), and these were used for accuracy determination.

Commercial bromhexine HCl preparations containing 48 mg 60 $mL^{-1}$ and 8 mg $tablet^{-1}$ were purchased in April 2003, from a local pharmacy in Surabaya (S-1, T-1, and T-2). All the commercial pharmaceutical preparations were produced in Indonesia.

Stock standard solutions were prepared daily by dissolving accurately weighed bromhexine hydrochloride (32.0 mg) in a mixture of acetone–water (2 : 1) 50 mL. Various standard solutions were prepared from the stock solution by dilution with a mixture of acetone–water (2 : 1). For linearity studies, solutions were prepared containing 32.0, 64.0, 106, 128, 160, 192, 213, 256, 284, and 320 μg $mL^{-1}$, and 5.0 μL of these solutions was spotted on the TLC plate.



## Sample Extraction

### Syrup

Two milliliter of the syrup was diluted with acetone–water (2 : 1) to 10.0 mL in a volumetric flask (10.0 mL). Five microliter of these solutions was spotted on the TLC plate.

### Tablets

Twenty tablets were each weighed and their mean was determined. After homogenizing the powder, an equivalent weight of 1/8 tablet (equivalent to 1.0 mg bromhexine hydrochloride) was transferred into a 10.0 mL volumetric flask containing about 9 mL of acetone–water (2 : 1), ultrasonicated for 15 min, and diluted to 10.0 mL with the mixture solution. The solution was filtered through Whatmann (Clifton, NJ) 41 filters before spotting on to TLC plates (5.0 μL).

### Chromatography

Chromatography was performed on pre-coated silica gel 60 $F_{254}$ TLC aluminum-backed sheets (E. Merck, Germany, #1.05554), which were cut into 10 × 20 cm sheets before use; a Nanomat III equipped with a dispenser magazine containing 5.0 μL glass capillaries (Cat. 022.7665; Camag,

Muttenz, Switzerland) was used for sample application. The mobile phase used was a mixture of *n*-butanol–glacial acetic acid–water (26 : 7.5 : 7.5). Ascending development was performed in a Camag twin-trough chamber (for 20 cm × 10 cm plates) after at least 3 hr of saturation; the mobile phase migration distance in all experiments was 8.0 cm (development time ca 90 min. at 23°C ± 2°C and room humidity 40% ± 10%). The $R_f$ of bromhexine hydrochloride was ca. 0.63.

Densitometric scanning was performed with a Camag TLC-Scanner II. The purity and identity of the analyte spots were determined by scanning in the absorbance–reflectance mode from 200 to 400 nm. Quantitative evaluation was performed by measuring the absorbance–reflectance of the analyte spots at $\lambda_{max}$ 325 nm (see Fig. 1). The densitometric scanning parameters were bandwidth 10 nm, slit width 4, slit length 6, and scanning speed 4 mm $sec^{-1}$. Calculations for identity, purity checks ($r_{S,M}$ and $r_{M,E}$, where S = start, M = center, E = end spectrum), sdv (relative standard deviation (RSD) of the linear/calibration curves), and quantification of the analyte spots were performed by CATS version 3.17 (1995) software (Camag). Routine quantitative evaluations were performed via peak areas with linear regression, using at least four-point calibration on each plate.



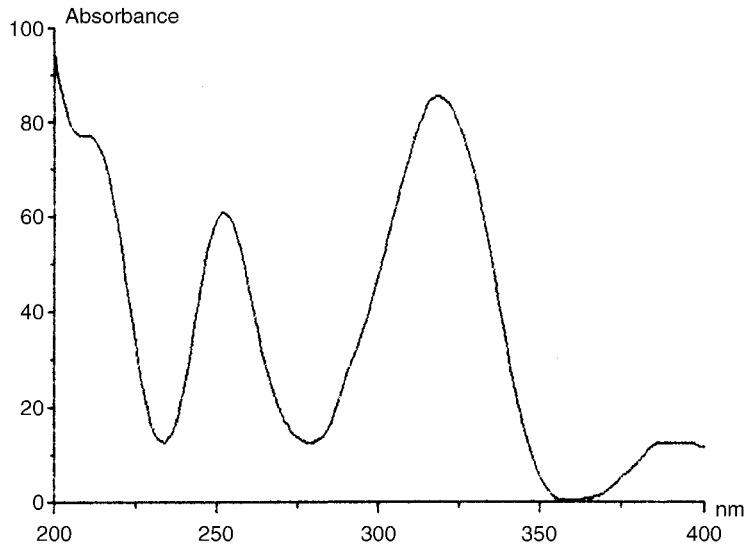


***Figure 1.*** In situ absorbance–reflectance spectrum of bromhexine hydrochloride from 200 to 400 nm, with its maximum absorption wavelengths at 250 and 325 nm. TLC conditions, stationary phase: pre-coated TLC plate silica gel 60 $F_{254}$ (E. Merck); mobile phase: a mixture of *n*-butanol–glacial acetic acid–water (26 : 7.5 : 7.5).

Validation

The method was validated for linearity, homogeneity, detection limit (DL), accuracy, and range by the modified method of Funk et al.[18] and Hahn-Dienstrop.[19] The selectivity of the method was proven by identification and purity checks of the analyte spots. A five-point accuracy study (80–120% of the expected value) was performed on the laboratory-made syrup preparations. For commercial preparations, accuracy studies were performed using a single-point standard addition method (with the addition of 40% of the label claim). The precision (repeatability and intermediate precision) was evaluated by analyzing six different extract aliquots from laboratory-made syrups containing bromhexine hydrochloride (38.4, 48.0, and 57.6 mg 60 $mL^{-1}$), according to the modified method of Renger et al.[20]



## RESULTS AND DISCUSSION

After the TLC plate was developed, the densitograms at 325 nm (Fig. 2) showed a single scan peak for the bromhexine hydrochloride zone ($R_f$ 0.63); the zones of the nipagin, nipasol, sodium cyclamate, and sodium saccharin were not detected. If the TLC plate was scanned at 250 nm (Fig. 3),

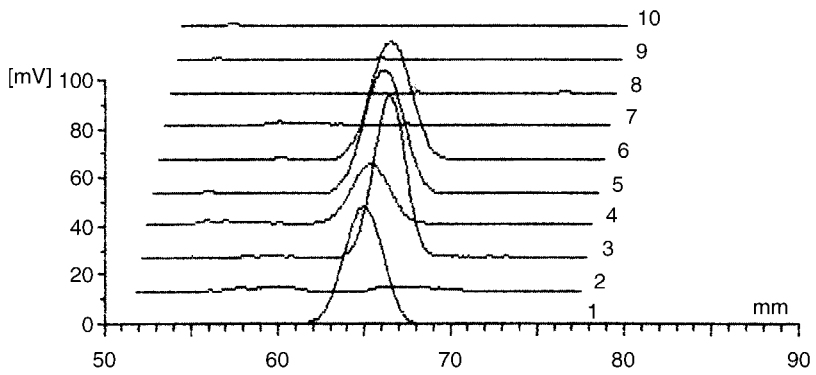


***Figure 2.*** Densitograms measured at 325 nm obtained from: (1) solution of standard bromhexine hydrochloride, 192 μg $mL^{-1}$; (2) extract from excipients of laboratory-made syrup; (3) extract of laboratory-made syrup; (4) extract of commercial syrup S-1; (5) extract of commercial tablet T-1; (6) extract of commercial tablet T-2; (7) solution of sodium saccharin, 600 μg $mL^{-1}$; (8) solution of sodium cyclamate, 200 μg $mL^{-1}$; (9) solution of nipagin, 200 μg $mL^{-1}$; and (10) solution of nipasol, 50 μg $mL^{-1}$. Spotted volumes were 5 μL. TLC conditions: see Fig. 1.

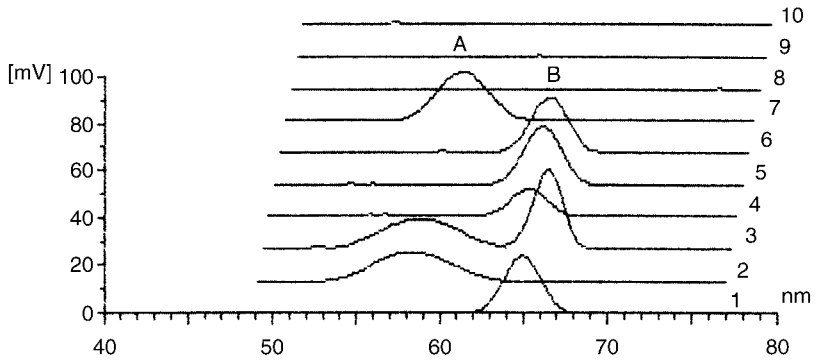


***Figure 3.*** Densitograms measured at 250 nm obtained from: (1) solution of standard bromhexine hydrochloride, 192 $\mu g\,mL^{-1}$, (2) extract from excipients of laboratory-made syrup, (3) extract of laboratory-made syrup, (4) extract of commercial syrup S-1, (5) extract of commercial tablet T-1, (6) extract of commercial tablet T-2, (7) solution of sodium saccharin, 600 $\mu g\,mL^{-1}$, (8) solution of sodium cyclamate, 200 $\mu g\,mL^{-1}$, (9) solution of nipagin, 200 $\mu g\,mL^{-1}$, and (10) solution of nipasol, 50 $\mu g\,mL^{-1}$. Spotted volumes were 5 $\mu L$. TLC conditions: see Fig. 1. Peak identities: (A) sodium saccharin and (B) bromhexine hydrochloride.



the scan of the bromhexine hydrochloride zone was well separated from the scan of sodium saccharin ($R_f$ 0.56), while other zones were also not detected. For further experiments, the TLC plates were scanned at 325 nm. In this TLC system, all the analyte spots of the laboratory-made syrup and commercial extracts furnished in situ UV absorption spectra identical to the standard ($r > 0.9999$). Purity checks using CATS software also showed that all of the analyte spots of the laboratory-made syrup and commercial extracts were pure. The values of $r_{S,M}$ and $r_{M,E}$ were $>0.9999$, demonstrating that the proposed TLC method is selective.

The basic calibration plot of peak area against amount of analyte was constructed within the range of 20–200% of the expected values in the pharmaceutical preparations. Under this condition, linearity of bromhexine hydrochloride was achieved from 160 to 1600 ng $spot^{-1}$ with the linear equation $Y = 51.81 + 1.49X$. The relative process standard deviation ($V_{XO}$) and $X_P$ values[18] of bromhexine hydrochloride were 2.36% and 92 ng $spot^{-1}$, respectively, ($n = 10$; sdv = 2.8; $r = 0.9991$). The ANOVA regression-test for determining linearity of the regression line showed a significant calculated $F$-value (4781.2 for $p < 0.0001$). The plots of the residuals against the quantities of the analyte confirmed the linearity of the basic calibration graphs (data not shown). The residuals were distributed at random around the regression line; neither trend nor uni-directional tendency was found. The basic

***Table 1.*** Results from determination of the accuracy of analysis of the laboratory-made (LM) and commercial preparations.

| Sample | Amount found[a] (mean ± SD)[b] | Amount added[a] | % Recovery (mean ± SD) | Recovery curve[c] | $V_{B(af)}$[d] | $V_{B(bf)}$[d] |
|---|---|---|---|---|---|---|
| LM-syrup | — | — | 101.4 ± 1.4[e] | $X_f = -42.6 + 1.07X_c$ | −42.6 ± 135.6 | 1.07 ± 0.17 |
| S-1 | 102.3 ± 1.3 | 40 | 100.5 ± 1.0[b] | — | — | — |
| T-1 | 93.2 ± 0.6 | 40 | 97.6 ± 0.4[b] | — | — | — |
| T-2 | 83.5 ± 0.8 | 40 | 99.1 ± 0.5[b] | — | — | — |

[a]Percentage of the label claim.
[b]$n = 3$.
[c]$X_f$ and $X_c$ are, respectively, the measured and nominal amount of the analyte spotted (ng spot$^{-1}$).
[d]$p = 0.05$.
[e]$n = 10$.

calibration curve showed variance homogeneity over the whole range. The calculated parameter PW[18] was 1.58. The PW-value was less from the $F_{table}$-value (5.35; for $f_1 = 9, f_2 = 9$; $\alpha = 0.01$).

Although, the validation parameters DL and quantitation limit (QL) were not required for the assay of active ingredient(s) in the pharmaceutical preparations,[21] those parameters were also determined in this present work. These parameters can be used for other purposes (e.g., for in vitro bio-equivalence-, stability-studies, etc.). DL was determined by making a linear regression of relatively low concentrations[18] of bromhexine hydrochloride ($32-160\,ng\,spot^{-1}$; $n = 5$; $V_{XO} = 1.84\%$; $r = 0.9995$; linear equation $Y =$
$78.2 + 11.7X$). An ANOVA regression-test showed a significant $F$-value (3295.7 for $p < 0.0001$). By this method, the calculated $X_p$[19] value was $11.7\,ng\,spot^{-1}$, and $DL = X_p$.[18] According to Carr and Wahlich,[22] the value of QL can be estimated as three times the DL-value ($35.1\,ng\,spot^{-1}$).

Table 1 demonstrates the relatively good accuracy, as revealed by the percentage of mean recovery data of the laboratory-made syrup (101.4%). To prove that systemic errors did not occur, linear regression of the recovery curve of $X_f$ (concentration of the analyte measured by the proposed method) against $X_c$ (nominal concentration of the analyte) of the laboratory-made syrup was constructed.[18] The confidence range data ($p = 0.05$) of the intercept $\{V_{B(af)}\}$ and slope $\{V_{B(bf)}\}$ from the recovery curves did not reveal the occurrence of constant- and proportional-systematic errors. Although, the results of the analysis of the commercial tablet preparations (T-1 and T-2) showed content of the active ingredient was below the required values in

***Table 2.*** Results from evaluation of precision of laboratory-made syrup.

| | RSD value ($n = 6$)[b] | | |
|---|---|---|---|
| Measurement[a] | Laboratory-made syrup A[c] | Laboratory-made syrup B[d] | Laboratory-made syrup C[e] |
| 1 | 0.99 | 1.55 | 1.52 |
| 2 | 1.35 | 1.65 | 1.70 |
| 3 | 1.80 | 1.76 | 1.66 |

[a]Each measurement was performed by a different analyst on different days and on different plates.
[b]Evaluated on one plate by one analyst (repeatability).
[c]Content of bromhexine HCl was $38.4\,mg\,60\,mL^{-1}$.
[d]Content of bromhexine HCl was $48.0\,mg\,60\,mL^{-1}$.
[e]Content of bromhexine HCl was $57.6\,mg\,60\,mL^{-1}$.

the Chinese Pharmacopoeia (93.0–107.0%)[5] and the Indian Pharmacopoeia (92.5–107.5%),[6] the results of accuracy studies using the standard addition method yielded relatively good results (Table 1). This showed that the stability of the commercial tablets T-1 and T-2 was not satisfactory.

All of the RSD values of the repeatability and intermediate precision evaluations were less than 2% (see Table 2). The three measurements were performed within one laboratory by different analysts on the different plates and days. These results demonstrated that the accuracy and precision of the proposed method were satisfactory in the range[19] of 80–120% of the expected value of the pharmaceutical preparation.

Therefore, the proposed method is suitable for the routine analysis of products of similar composition in pharmaceutical industry quality control laboratories.



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