

Short communication

Simultaneous assay of phenylpropanolamine hydrochloride, caffeine, paracetamol, glycerylguaiacolate and chlorpheniramine maleate in Silabat<sup>TM</sup> tablet using HPLC with diode array detection

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## 1. Introduction

Silabat<sup>TM</sup> is an over-the-counter cold tablet medication produced in Indonesia by PT Bernofarm Pharmaceutical Industry, Surabaya, Indonesia. One Silabat tablet contained the following active drug substances including 25 mg phenylpropanolamine HCl (PPA), 30 mg caffeine (CAF), 500 mg paracetamol (PAR), 25 mg glycerylguaiacolate (GGL) and 2 mg chlorpheniramine maleate (CTM). Methods exist in the literature for the assay of one or more, but not for all of the Silabat active components [1–4]. The analysis of Silabat was further complicated by the presence of relatively high PAR concentration compared to the other components. Analysis of relatively high concentration of PAR in the mixture with low concentration of PPA and GGL required two separate isocratic high-performance liquid chromatography (HPLC) (with conventional UV-detector) systems [5].

In the present work, simultaneous assay of PPA, CAF, PAR, GGL and CTM in Silabat using high-performance liquid chromatography

(HPLC) equipped with a diode array detector has been developed.

## 2. Experimental

### 2.1. Equipment

The HPLC system was comprised of a Hitachi L-6200 intelligent pump equipped with a Hitachi LC organizer and dynamic mixer mode 655A, a Hitachi L-4500 photo diode array (PDA) detector and a 20  $\mu$ l Rheodyne 7125 injector. The analysis was carried out on a LiChrospher 100 CN (5  $\mu$ m) column (E. Merck, Cat. No. 50892). All data evaluations (identification, purity check and quantitation) were performed using a Hitachi model D-6500 chromatography data station software, DAD system manager.

### 2.2. Materials and reagents

Acetonitrile (ACN), methanol (MeOH), tetrahydrofuran (THF) and hexanesulphonic acid sodium salt (HSA) were Hiper-Solv for HPLC from BDH Chemicals. Di-*n*-butylamine (DBA) was GPR grade from BDH Chemicals, acetic acid glacial (AA) was Baker Analyzed

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reagent (J.T. Baker), and phosphoric acid (PA) was analytical grade reagent (E. Merck). PAR (Xian-Janssen Pharmaceutical, China), PPA (Alps Pharmaceutical, Japan), CAF (Yang Zhou San Pharmaceutical, China), GGL (Rhone Poulenc, France) and CTM (Kongo Chemical, Japan) were pharmaceutical grade. Sodium maleate, PAR, PPA, CAF, GGL and CTM for standards were from Sigma. Excipients for Silabat tablet (avicel PH101, primojel, magnesium stearate, talc, aerosil and PVP) were pharmaceutical grade.

### 2.3. Chromatography

A modified mobile phase of previous work [5] consisting of a mixture of ACN-ion pair solution (15:85, v/v) (pH\* 3.3) was used for initial separation. The ion pair solution contained an aqueous solution of HSA (5 mM), DBA (10 mM), AA (0.8% v/v) and PA (0.12% v/v). The flow rate was 1 ml min<sup>-1</sup>. The mobile phase was further optimized using the triangle method [6]. The mobile phase was prepared daily, the filtered through to 0.45 µm Millipore filter and ultrasonicated for 30 min before use.

The eluent was monitored by PDA detector in the range of 210–400 nm. Identification, purity check of the analyte peaks and quantitative evaluations were achieved using authentic standards. Quantitations were performed at 260 nm (for PPA), 298 nm (CAF), 310 nm (PAR), 284 nm (GGL) and 265 nm (CTM).

### 2.4. Preparations of standard solutions

195.0 mg PPA and 31.2 mg CTM were accurately weighed and dissolved with the mobile phase up to volume in 25 ml volumetric flask (solution A). 45.0 mg CAF, 750.0 mg PAR and 37.5 mg GGL were accurately weighed, mixed and dissolved with 20.0 ml of mobile phase in a 25 ml volumetric flask. 2.5 ml of solution A was then added to this solution and made up to volume with the mobile phase. Various standard solutions were prepared from this stock solution after adequate dilution with the mobile phase and filtered through 0.2 µm Millipore filters prior to injection.

### 2.5. Sample preparation

Twenty Silabat tablets were weighed and their mean weight determined. The tablets were

finely powdered, and an equivalent weight of a half tablet was transferred into a 10 ml volumetric flask containing about 9 ml of the mobile phase, ultrasonicated for 20 min and diluted to 10 ml with the mobile phase. The solution was filtered through Whatman type 40 and 0.2 µm Millipore filters before injection to HPLC apparatus.

### 2.6. Validation

The method was validated for linearity, limit of detection (LOD), accuracy and precision according to the method of Funk et al. [7]. The selectivity of the method was proved by identification and purity check of the analyte peaks. For accuracy determination, a five point recovery study of Silabat tablet was performed at 60, 80, 100, 120 and 140% level of its original formulation. The precision was evaluated by analyzing 10 different aliquots from Silabat tablets at the original concentration.

## 3. Results and discussion

When a mixture of ACN-ion pair solution (15:85, v/v) (pH\* 3.3) was used, the peaks of CAF and PAR were unresolved (Fig. 1(A)). In this mobile phase CTM was separated into two peaks, chlorpheniramine (CH) and maleic acid (M). The identification of the peaks was confirmed by coelution with standard sodium maleate and comparison of its UV-spectra with the standards. In order to get a good separation of PAR and CAF, a mobile phase optimization triangle method was used [6]. Separation of the five components of Silabat was achieved using a mixture of ACN-THF-ion pair solution (7:6:87, v/v/v) (pH\* 3.3). Thus, this eluent was used for further work (Fig. 2(A)).

Owing to the relatively low concentration of CTM and relatively small response factor of PPA, the  $\lambda_{\max}$  of CTM (265 nm) and PPA (260 nm) were selected for quantitative work. However, the assay of PAR was performed at 310 nm owing to its relatively high concentration. The absorbance of CAF and PAR exceeded the linear range of the detector at their respective  $\lambda_{\max}$  values. Using five different wavelengths for detection, a good chromatogram for quantitative work was obtained (Fig. 2(B)). Therefore, two HPLC analyses of a mixture with a relatively high concentration of

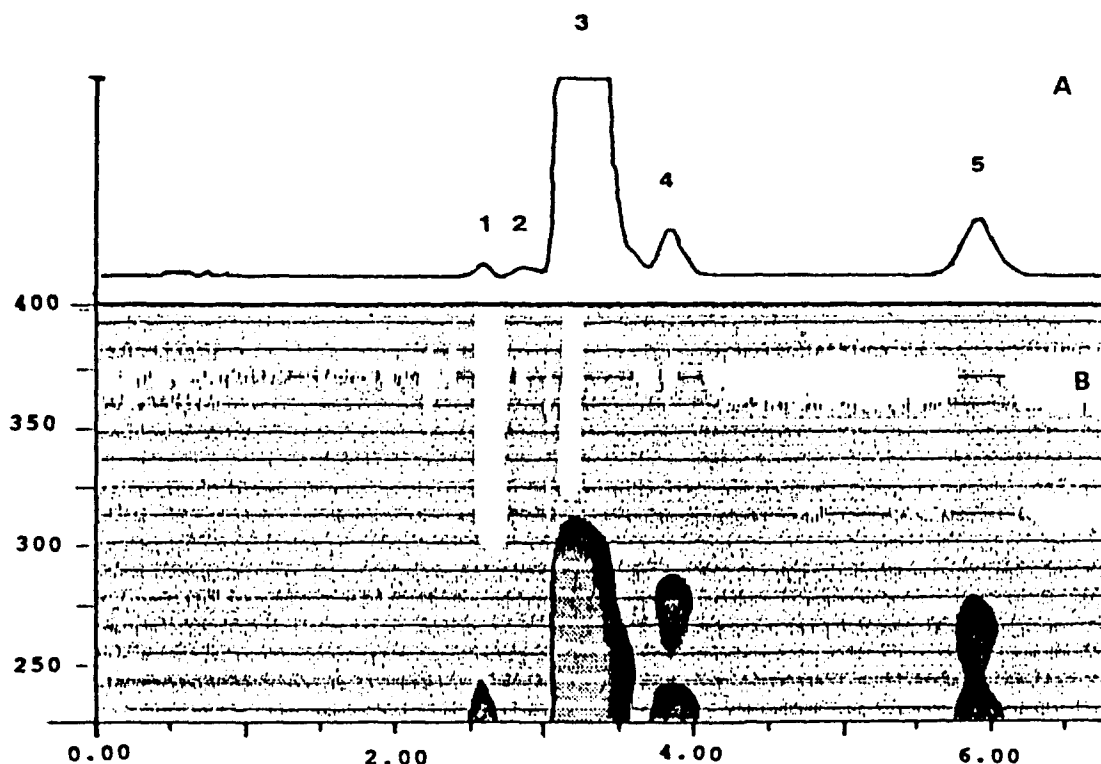


Fig. 1. (A) A typical HPLC chromatogram of Silbat tablet using ACN-ion pair solution (15:85, v/v) (pH\* 3.3) as mobile phase. Peaks were identified as: 1 (M); 2 (PPA); (CAF and PAR); 4 (GGL) and 5 (CTM). Vertical axis: detector response at 260 nm. Horizontal axis: retention time in min. (B) Contour plot of HPLC chromatogram from 210 to 400 nm. Vertical axis: wavelength (nm). Horizontal axis: retention time (min).

PAR containing low concentrations of other ingredients could be avoided. Using a PDA detector, the identity and purity of the analyte peaks could be also determined. All the UV-spectra of the analyte peaks showed good correlations with the standards ( $r > 0.99$ ). A purity check of the analyte peaks showed that all the peaks were pure ( $r > 0.99$ ). A peak contaminated by the excipients could lead to a wrong result of the analysis [8]. Identification and purity check of the analyte peaks are impossible using HPLC apparatus equipped with a conventional UV-detector only.

Using the mobile phase consisting of ACN-THF-ion pair solution (7:6:87, v/v/v) (pH\* 3.3), linearity was achieved for: 156.0–1264.0  $\mu\text{g/ml}^{-1}$ ,  $r = 0.9997$ ,  $n = 5$  (PPA); 276.0–3020.0  $\mu\text{g ml}^{-1}$ ,  $r = 0.9995$ ,  $n = 5$  (CAF); 4084.0–46160.0  $\mu\text{g ml}^{-1}$ ,  $r = 0.9997$ ,  $n = 5$  (PAR); 213.0–2515.0  $\mu\text{g ml}^{-1}$ ,  $r = 0.9994$ ,  $n = 5$  (GGL); 25.0–199.7  $\mu\text{g ml}^{-1}$ ,  $r = 0.9999$ ,  $n = 5$  (CTM). The linearity of all components was also proved by Mandel's test according to Funk et al. [7]. LOD = 15.1  $\mu\text{g ml}^{-1}$  (PPA); 1.1  $\mu\text{g ml}^{-1}$  (CAF); 1.9  $\mu\text{g ml}^{-1}$  (PAR); 2.8  $\mu\text{g ml}^{-1}$  (GGL); 2.9  $\mu\text{g ml}^{-1}$

(CTM). According to Carr and Wahlich [9] the limit of quantitation could be estimated as 3.3 times the LOD-values.

Table 1 showed good accuracy as revealed by the percentage of mean recovery data (98.5–101.8%). To prove whether systematic errors occurred, linear regression accuracy curves of  $X_r$  (measured concentration of the analyte by the proposed method) vs.  $X_c$  (nominal concentration of the analyte) were constructed [7]. The confidence range data ( $p < 0.05$ ) of the intercept ( $V_b a_i$ ) and slope ( $V_b b_i$ ) from the accuracy curves did not show the occurrence of constant and concentration dependent systematic errors [7].

The RSD of 10 replicate analyses of Silabat tablets were 1.12% (PPA), 1.11% (CAF), 1.09% (PAR), 1.07% (GGL) and 1.30% (CTM). All the RSD values were less than 2%.

#### 4. Conclusions

A simple, selective, rapid, accurate and precise HPLC method for simultaneous determination of PPA, CAF, PAR, GGL and CTM in

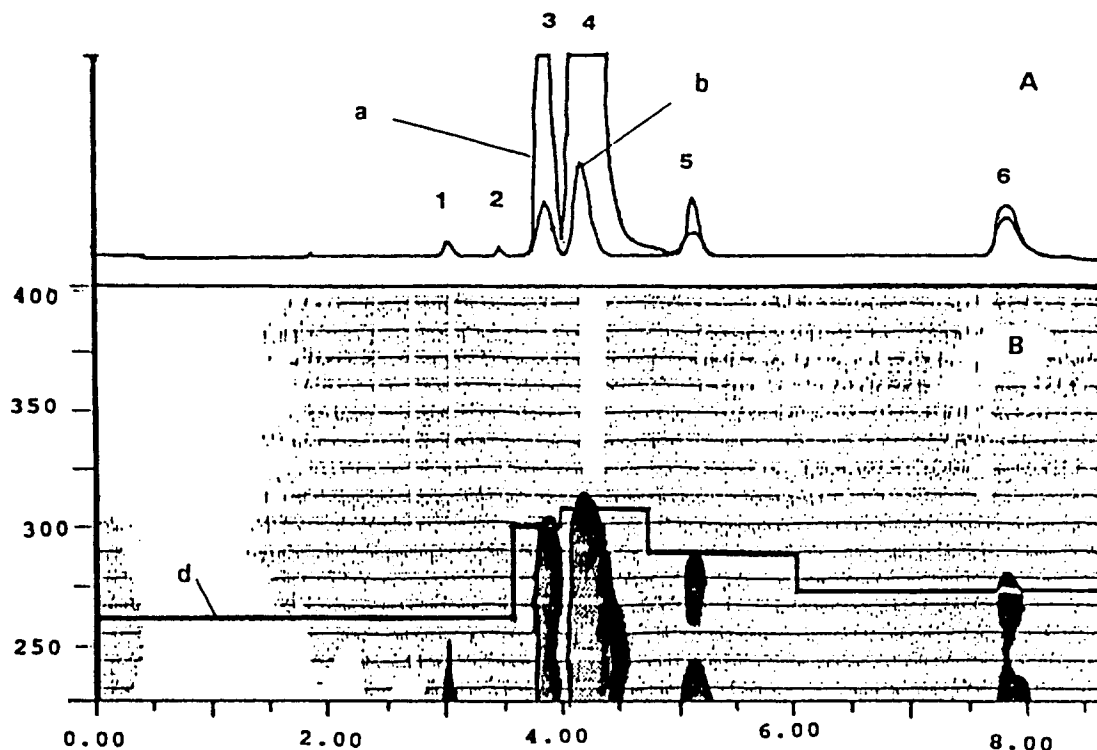


Fig. 2. (A) A typical HPLC chromatogram of Silabat tablet using ACN–THF-ion pair solution (7:6:87, v/v/v) (pH\* 3.3) as mobile phase. Peaks were identified as: 1 (M); 2 (PPA); 3 (CAF); 4 (PAR); 5 (GGL) and 6 (CH). Vertical axis: detector response at 260 nm (a) and detector response using five different wavelengths for quantitative determination (b). Horizontal axis: retention time. (B) Contour plot of HPLC chromatogram from 210 to 400 nm. (d) detection wavelength for quantitative determination. Vertical axis: wavelength (nm). Horizontal axis: retention time (min).

Table 1  
The results of accuracy determination of Silabat<sup>TM</sup> tablet

Analyte	Recovery (%) mean $\pm$ SD ( $n = 5$ )	Accuracy curve $X_r = a_r + b_r X_c$ <sup>c</sup>	$V_b a_r$ <sup>a</sup>	$V_b b_r$ <sup>b</sup>
PPA	101.5 $\pm$ 2.2	$X_r = 83.0 + 0.89 X_c$	83.0 $\pm$ 139.6	0.89 $\pm$ 0.20
CAF	101.8 $\pm$ 2.5	$X_r = 97.5 + 0.97 X_c$	97.5 $\pm$ 170.3	0.97 $\pm$ 0.11
PAR	100.8 $\pm$ 1.4	$X_r = 185.9 + 1.00 X_c$	185.9 $\pm$ 289.8	1.00 $\pm$ 0.11
GGL	99.1 $\pm$ 2.1	$X_r = 49.6 + 0.95 X_c$	49.6 $\pm$ 159.4	0.95 $\pm$ 0.12
CTM	98.5 $\pm$ 1.3	$X_r = -0.64 + 0.98 X_c$	-0.6 $\pm$ 4.7	0.98 $\pm$ 4.42

<sup>a</sup> Confidence range of the intercept,  $a_r$  ( $p < 0.05$ ).

<sup>b</sup> Confidence range of the slope,  $b_r$  ( $p < 0.05$ ).

<sup>c</sup>  $X_r$  = measured concentration of the analyte in the injected solution ( $\mu\text{g ml}^{-1}$ ),  $X_c$  = nominal concentration of the analyte in the injected solution ( $\mu\text{g ml}^{-1}$ ).

Silabat tablets has been developed. This method has been found suitable for the routine analysis of the tablets in quality control and R&D laboratories for products of similar type and composition.

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