



# Comprehensive evaluation of extemporaneous preparation containing ambroxol HCl and salbutamol sulfate: Compatibility, chemometrics, and stability study

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## ABSTRACT

Extemporaneous compounding preparations of divided powder were commonly prescribed in Indonesia. Since it is important to implement the Good Pharmacy Practices Guideline, the compounded preparations should be evaluated for several quality parameters. This study aimed to perform a comprehensive evaluation of extemporaneous compounding preparation containing ambroxol HCl and salbutamol sulfate, including compatibility, chemometrics, and stability study. A compatibility study was carried out by evaluating FTIR spectroscopy on functional group change during the interaction between drugs and excipients. A chemometric approach of multivariate calibration was conducted to build predictive content determination for ambroxol HCl and salbutamol sulfate. It was found that the selected models for ambroxol HCl and salbutamol sulfate were partial least squares (PLS) on second derivative spectra and PLS on Savitzky–Golay spectra, respectively. The equation of multivariate calibration for ambroxol HCl was  $y = 0.975x - 0.089$  ( $R_{val}^2 = 0.989$ ), whereas the equation of multivariate calibration for salbutamol sulfate was  $y = 0.970x - 0.119$  ( $R_{val}^2 = 0.943$ ). These models were employed for content determination in the stability study. Divided powder preparation samples were stable during seven days of storage.

## INTRODUCTION

Extemporaneous compounding practices remain in high demand in Indonesia, especially in the pediatric population (Virginia, 2014). Notably, compounding is regulated in Indonesia through the Good Pharmacy Practices Guideline (Kementerian Kesehatan Republik Indonesia & Pengurus Pusat Ikatan Apoteker Indonesia, 2011). The pharmacist is a healthcare professional ensuring the quality of extemporaneous compounding products (International Pharmaceutical Federation, 2020; Mohiuddin, 2019). A previous

case report, specifically in the divided powder compounding area, explored that the most prescribed formula tends to increase the risk of instability and incompatibility (Yuliani *et al.*, 2020).

Our study focused on divided powder preparation (DPP) containing ambroxol HCl and salbutamol sulfate as one of the most regularly prescribed. The randomized controlled trial found that the combination of ambroxol HCl 15 mg and salbutamol 4 mg does not have pharmacokinetic interactions. Thereby, it ensures the safety of those combination formulas (Wang *et al.*, 2018). Ambroxol is an over-the-counter medicine which has been proven effective and well tolerated in pediatrics as a mucoactive agent (Kantar *et al.*, 2020). On the other hand, salbutamol is a bronchodilator where inappropriate concentration induces electrolyte disturbance in children. Therefore, compounding practice should be able to minimize the risk of ambroxol/salbutamol concentration/dose error due to incompatibility and/or instability.

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The pharmacist is responsible for preventing pharmaceutical incompatibility, either physical or chemical (Mohiuddin, 2019; Yarnykh *et al.*, 2020). A previous study carried out by Dijkers *et al.* (2017) found that there were no significant effects of excipient to incompatibility according to beyond use date of extemporaneous preparation. Nonetheless, limited study has been observed related to compatibility in divided powder preparation, especially in pharmacy practice in Indonesia.

Drug stability could be determined through drug concentration and purity of formulated products (Bajaj *et al.*, 2012). Storage conditions affect the physical stability of pharmaceutical products (Winter *et al.*, 2013). Interaction between drug and excipient could induce instability of drug products. Thus, it could affect the effectiveness and safety of active ingredients (Darji *et al.*, 2017; Murthy and Repka, 2017). Limited study related to the possible drug–excipient interaction and lack of mathematical modeling to predict stability should be pivotal consideration (Dave *et al.*, 2015; Tamura *et al.*, 2020), especially in extemporaneous preparations. The UV spectrophotometric method assists in detecting drug concentration as a drug stability indicator (Chakraborty *et al.*, 2018). The combination of the UV spectrophotometric method and chemometrics could produce an adequate quantitative analysis.

Chemometrics is an effective and promising mathematical tool to predict and classify drug concentration in pharmaceutical formulas (Biancolillo and Marini, 2018; Singh *et al.*, 2013). A previous study showed chemometric success in determining paracetamol and tramadol concentration divided powder dosage form (Putri *et al.*, 2020). The other study confirmed that chemometrics is a favorable tool for figuring out the ingredients of tablets containing acetaminophen, caffeine, and propyphenazone (Rohman *et al.*, 2017). Accordingly, this present study applied chemometric analysis to predict ambroxol and salbutamol concentration after being compounded into divided powder dosage forms.

A private hospital in Semarang, Indonesia, reported that they did compounding practice with the highest preparation being ambroxol and salbutamol sulfate. However, the quality, incompatibility, and stability of extemporaneous preparation were still undiscovered in that hospital. Therefore, this study aimed to explore the stability and compatibility of ambroxol and salbutamol as extemporaneous products and to assess the chemometric application predicting the ambroxol and salbutamol concentration.

## METHODS

### Materials

The ambroxol HCl standard was obtained from PT IFARS (Solo, Indonesia). The salbutamol sulfate standard was obtained from PT Dexa Medica (Palembang, Indonesia). The solvent used in this study was methanol pro analysis (Smartlab, Indonesia), ambroxol tablets (containing ambroxol HCl 30 mg manufactured by PT. Triman, Indonesia), salbutamol tablets (containing salbutamol sulfate 4 mg manufactured by PT. Mulia Farma, Indonesia), and DPP sample containing ambroxol HCl and salbutamol sulfate were prepared and formulated by a pharmacist in the pharmacy installation of the private hospital, Indonesia.

### Instrumentation and software

The instruments used in this study were a UV 1800 UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan) equipped with a 1 cm quartz cuvette (Hellma, Germany), an FTIR model IRSpirit-T (Shimadzu, Kyoto, Japan), a climatic chamber (Mettler, Germany), an analytical balance model PA224C with specification max, 220 g; minute, 0.02 g (Ohaus®, USA), and a set of Socorex® (Eublens, Switzerland) micropipettes. UV and FTIR spectral acquisition were controlled and processed using UV Probe and Lab Solution R software (Shimadzu, Japan), respectively.

Multivariate calibrations and spectral preprocessing were executed using R statistical software version 4.1.1 with “pls” and “prospectr” packages, respectively (Mevik and Wehrens, 2019). The UV spectral data obtained from the spectrophotometer instruments were exported to Excel 2016 (Microsoft Inc., Redmond, WA) and converted into the format files of .csv. for further stage of data analysis.

### Sample preparation for quality test

The samples of DPP were obtained from the hospital pharmacy department with the following prescription:

R/ Salbutamol 4 mg tablet ½  
 Ambroxol 30 mg tablet ½  
 M f pulv. dtd no X  
 S t d d l

The sample preparation was replicated 6 times so that a total of 60 packs of preparations were obtained. The samples were divided for various tests, including 10 packs for organoleptic testing, 10 packs for moisture content testing, 10 packs for micromeritic testing, and 30 packs for content uniformity testing.

### Sample preparation for stability and compatibility test

The samples for stability and compatibility test were prepared by weighing the crushed tablet of ambroxol equivalent to 15 mg of ambroxol HCl and the crushed tablet of salbutamol sulfate equivalent to 2 mg of salbutamol sulfate; then, both were mixed to obtain one pack. The processes were repeated until a total of 20 packs were obtained for stability and compatibility evaluations. The samples were stored at room temperature with a maximum of 75% RH.

### Physical properties

The physical properties of DPP were observed by the organoleptic tests (smell, shape, color, and visual homogeneity), moisture content test, and micromeritic test. The organoleptic test was carried out visually and subjectively by the researcher. The content test was carried out on DPP using a moisture balance tool to determine the moisture content. The micromeritic test was carried out by dispersing the extemporaneous preparation in distilled water and then observing under a microscope. Particle measurement was done using ImageJ software.

### Calibration and validation solutions

The standard solutions of ambroxol HCl and salbutamol sulfate were each prepared using methanol as a solvent with a concentration of 1,000 ppm. From the main standard solution, a

calibration and validation set solution was then prepared with the concentration variations listed in Table 1.

### Chemometrics study

All prepared solutions were scanned using the UV-Vis spectrophotometer type of UV 1800 (Shimadzu, Japan) at the wavelength range of 240–350 nm (interval of 2 nm). The absorbance values of every single wavelength point achieved from the scanning process were collected and processed using the R statistical software. Absorbance data of calibration and validation solution were preprocessed into five types of UV-Vis spectra, including normal/original, first derivative, second derivative, standard normal variate (SNV), and Savitzky–Golay (SG) smoothing (window width of 11 points, polynomial order of 3). Two calibration models of principal component regression (PCR) and PLS were built using all types of spectra. The best multivariate calibration model for each compound was selected according to several chemometric parameters such as the coefficient of determination for calibration ( $R_{cal}^2$ ), cross-validation ( $R_{CV}^2$ ), validation ( $R_{val}^2$ ), root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV), and root mean square error of prediction (RMSEP).

### FTIR spectral scanning

The sample was homogenized before this study. Spectral scanning was carried out by placing homogenized powder of sample (ambroxol HCl standard, salbutamol sulfate standard, and mixture of standard and sample of DPP containing ambroxol HCl and salbutamol sulfate from private hospital) into a compartment for sample in the instrument. It was scanned in the FTIR spectrophotometer in the transmittance mode. The data were recorded at wavelength range 4,000–400  $\text{cm}^{-1}$ .

### Stability study

A stability study was carried out by a stored sample of DPP preparation containing ambroxol HCl and salbutamol sulfate from the private hospital in a climatic chamber (30°C; RH 75%) for 1, 7, and 14 days in this study. On the day of the study, three replicate samples were weighed. Each sample was prepared by dissolving in methanol, put in a 50.0 mL volumetric flask, and added with methanol until the calibration mark. 500 microliters was taken out and added to a 5.0 mL volumetric flask and added with methanol until the calibration mark. The solution of the sample was scanned in the UV spectrophotometer in the lambda 240–350 nm with the interval scanning of 2 nm.

**Table 1.** Series of calibration and validation solutions for each compound.

No.	Variation of calibration solution (ppm)		Variation of validation solution (ppm)		
	Ambroxol HCl	Salbutamol sulfate	Ambroxol HCl	Salbutamol sulfate	
1.	11	8	1.	10	7
2.	10	3	2.	22	3
3.	7	7	3.	9	7
4.	20	2	4.	5	5
5.	10	6	5.	9	1
6.	25	8	6.	11	10
7.	19	1	7.	17	2
8.	17	3	8.	7	9
9.	18	9	9.	7	2
10.	23	2	10.	9	8
11.	24	1			
12.	25	7			
13.	15	10			
14.	19	3			
15.	17	9			
16.	9	2			
17.	5	7			
18.	21	7			
19.	14	10			
20.	16	3			
21.	11	8			
22.	24	10			
23.	23	9			
24.	8	2			
25.	9	9			

## RESULTS AND DISCUSSION

The evaluation of the quality of the DPP compounded by the hospital pharmacy was started with an organoleptic test. Observations were carried out on the physical appearance of the sample and the homogeneity of the mixture visually. The DPPs had a visual appearance as white, homogeneous, and odorless powder (Fig. 1).

The moisture content of the DPP was analyzed on the first and seventh days of storage. The mean of moisture content on the first day was 6.04% and on the seventh day was 6.65%. The moisture content increased during the storage of the DPP. The powder should have a moisture content of less than 5% to prevent drug degradation through hydrolysis mechanisms or due to microbial contamination.

Evaluations on particle size were carried out to determine the level of fineness and uniformity of the size of the samples. Extemporaneous preparation in powder dosage form has to be fine and have a uniform particle size. The results of measurements of 300 powder particles showed the average diameter of the particle size was 18.33 micrometers. The particle size of the DPPs was in the range of 10.09–29.93  $\mu\text{m}$  with a mode value of 17.03  $\mu\text{m}$ . The profile of the particle distribution is shown in Figure 2.

### Compatibility study

A compatibility study was carried out by assessing the FTIR spectra of the DPP sample containing ambroxol HCl and salbutamol sulfate as well as ambroxol HCl and salbutamol sulfate standard. FTIR spectroscopy was applied in the compatibility study since it was stated as an effective technique to examine the compatibility of the drug according to the same functional group change during the interaction between drugs and excipients (Rojek and Wesolowski, 2019). The location and structure of the functional groups found in the FTIR spectra of pure drug and drug–excipient mixtures were evaluated and compared to each other. The band shifting and widening in the spectra indicated the interaction between the active drug and excipients (Seçilmiş Canbay *et al.*, 2019; Swathi and Reddy, 2016). Figure 3 shows the FTIR spectra for ambroxol HCl standard, salbutamol sulfate standard, the mixture of ambroxol HCl and salbutamol sulfate, and the sample of DPP preparation containing ambroxol HCl and salbutamol sulfate.

The FTIR spectra of ambroxol HCl were characterized by several peaks at 3,450–3,375  $\text{cm}^{-1}$ , 3,350–3,100  $\text{cm}^{-1}$ , 1,300–1,100  $\text{cm}^{-1}$ , and 700–600  $\text{cm}^{-1}$  corresponding to the presence of functional groups such as intermolecular hydrogen-bonded OH

(stretching), aromatic primary amine (NH stretching), aromatic C=C (stretching), and bromo compound (C-Br stretching), respectively (Daharwal *et al.*, 2013). The FTIR spectra of salbutamol sulfate showed intense absorption bands at 2,900, 1,380, 1,200, and 1,100  $\text{cm}^{-1}$  corresponding to the presence of functional groups such as secondary amine (N-H stretching), tertiary carbon (C-H bending), phenolic group (C-O stretching), and primary alcohol (C-O stretching), respectively (Sharma *et al.*, 2015). The laboratory preparation of the ambroxol HCl and salbutamol sulfate mixture was also compared to the sample of extemporaneous compounding preparation containing ambroxol HCl and salbutamol sulfate. These FTIR spectra showed similar profiles with few variations at the wavenumber range of 900–600  $\text{cm}^{-1}$  indicating the presence of drug excipients (Aminu *et al.*, 2021). However, the FTIR profiles of the sample showed were similar compared to the FTIR spectra of a fixed-dose combination containing ambroxol hydrochloride and salbutamol sulfate prepared by direct compression in the previous study (Sharma *et al.*, 2018).

The presence of the broad band of absorption at 1,600–1,550  $\text{cm}^{-1}$  and 1,450–1,400  $\text{cm}^{-1}$  in the FTIR spectra of ambroxol HCl standard indicated the aromatic (C=C stretching) and aliphatic (C-H bending) functional groups, respectively. These peaks were identified as unknown impurity peaks commonly found as a degradation product of ambroxol HCl which has been formed due to several factors such as heat, light, and moisture during storage (Thummala *et al.*, 2014). According to our limitations, only the FTIR method was evaluated in this compatibility study. Since there were two types of chemical incompatibilities including

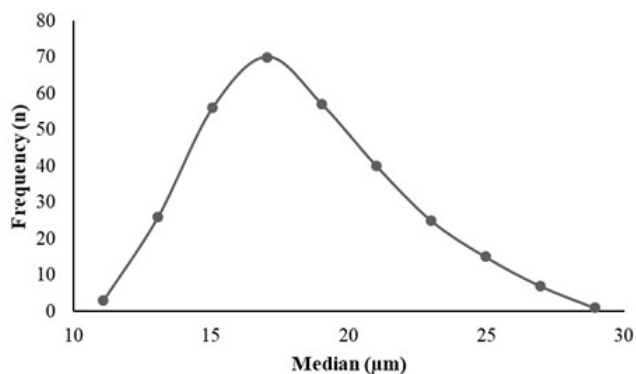


Figure 2. Particle size distribution curve of DPP samples.

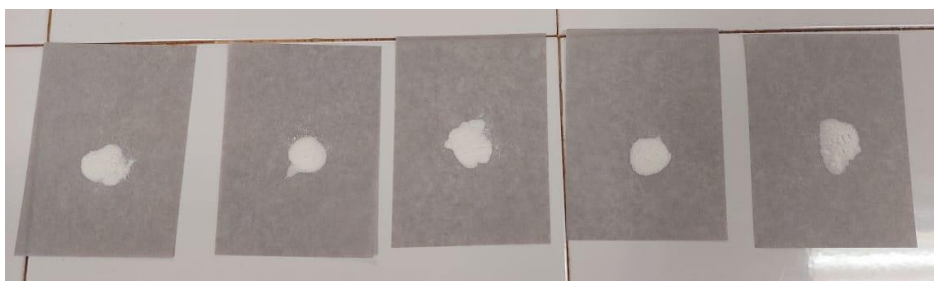
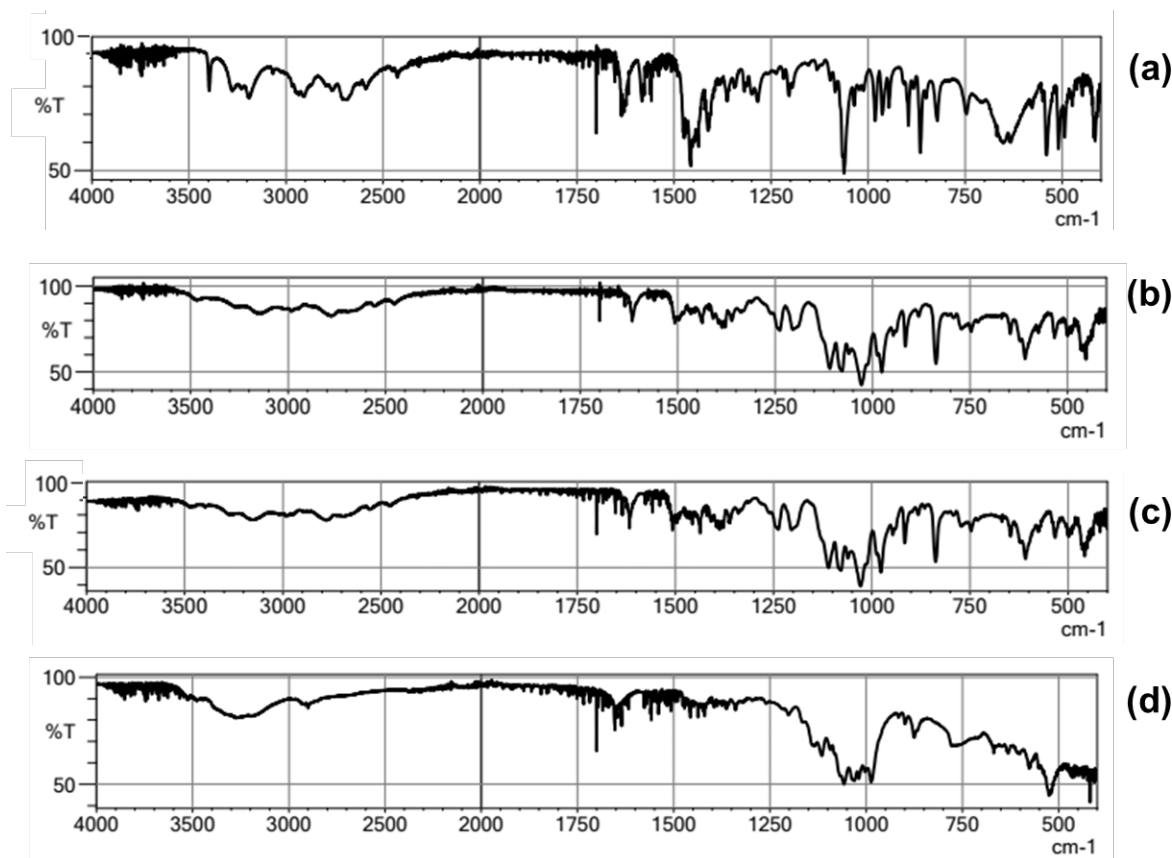


Figure 1. Representative appearance of DPP samples.





**Figure 3.** FTIR spectra for (a) ambroxol HCl, (b) salbutamol sulfate, (c) mixture of ambroxol HCl and salbutamol sulfate, (d) and sample of extemporaneous compounding preparation containing ambroxol HCl and salbutamol sulfate.

excipient-induced structural degradation of the drug and covalent reaction between the drug and the excipient (Seçilmiş Canbay *et al.*, 2019), it was important to perform combination techniques in order to evaluate the compatibility of different pharmaceutical excipients such as FTIR, differential scanning calorimetric, and isothermal stress testing in the future (Rojek and Wesolowski, 2019; Jeličić *et al.*, 2021).

### Chemometrics study

The chemometrics techniques of multivariate calibration were implemented in this study. Two multivariate calibration techniques, namely, PCR and PLS, were applied to generate predictive models for both ambroxol HCl and salbutamol sulfate. Spectral preprocessing techniques were carried out to obtain the various types of spectra such as original, first derivative, second derivative, SNV, and SG. The quality of the multivariate calibration models was evaluated according to the lowest value of RMSEC and RMSEP as well as the highest value of  $R_{cal}^2$  and  $R_{val}^2$ . Since the cross-validation of the multivariate calibration models was also performed using the leave-one-out technique, the lowest value of RMSECV and the highest value of  $R_{cv}^2$  were considered to select the appropriate model for each compound. Table 2 presents the performance of PCR and PLS for predicting the content of ambroxol HCl and salbutamol sulfate.

According to the presented data in Table 2, it was found that the selected model for ambroxol HCl and salbutamol

sulfate was PLS on second derivative spectra and PLS on SG spectra, respectively. These models were stated as the selected model since their highest  $R^2$  ( $R_{cal}^2$ ,  $R_{val}^2$ , and  $R_{cv}^2$ ) represented the smaller differences between the actual and calculated values, and their lowest RMSE (RMSEC, RMSEP, and RMSECV) indicated a better fit with the minimum errors resulting from the models (Riswanto *et al.*, 2021).

Figure 4 shows the regression coefficient plots and multivariate regression plots for ambroxol HCl and salbutamol sulfate. The important wavelength to generate multivariate calibration models of ambroxol HCl indicated by the presence of an extensive peak and trough in the regression coefficient plots was located in the range of 240–278 nm, whereas the important wavelength to generate multivariate calibration models of salbutamol sulfate was in the range of 240–298 nm. The equation for correlating between the actual and the predicted values of ambroxol HCl was  $y = 0.975x - 0.089$  ( $R^2 = 0.989$ ). The equation for correlating between the actual and the predicted values of salbutamol sulfate was  $y = 0.970x - 0.119$  ( $R^2 = 0.943$ ). These equations were used in the stability study in order to quantitatively analyze the content of ambroxol HCl and salbutamol sulfate in the samples.

### Stability study

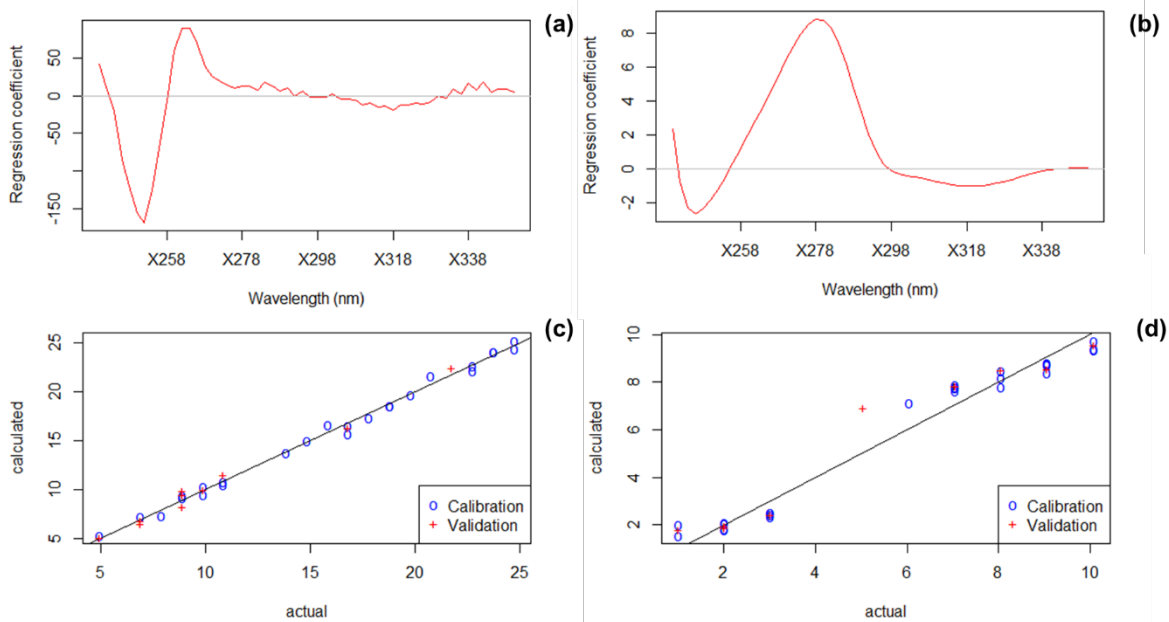
A stability study was carried out by calculating the actual value content of DPP containing ambroxol HCl and

**Table 2.** The performance of PCR and PLS for predicting the content of ambroxol HCl and salbutamol sulfate.

Analytes	Multivariate calibration	Type of spectra	Number of components	$R_{cal}^2$	RMSEC	$R_{CV}^2$	RMSECV	$R_{val}^2$	RMSEP
Ambroxol HCl	PCR	Original	11	0.997	0.311	0.993	0.501	0.981	0.668
		First derivative	5	0.995	0.437	0.993	0.514	0.988	0.539
		Second derivative	3	0.995	0.447	0.993	0.517	0.988	0.525
		SNV	21	0.998	0.243	0.955	1.291	0.954	1.028
		SG	12	0.998	0.283	0.993	0.519	0.975	0.759
	PLS	Original	5	0.997	0.340	0.993	0.523	0.979	0.697
		First derivative	2	0.994	0.455	0.993	0.515	0.988	0.530
		<b>Second derivative</b>	<b>2</b>	<b>0.994</b>	<b>0.456</b>	<b>0.993</b>	<b>0.521</b>	<b>0.989</b>	<b>0.515</b>
		SNV	7	0.994	0.469	0.953	1.314	0.958	0.988
		SG	4	0.995	0.412	0.992	0.531	0.976	0.732
Salbutamol sulfate	PCR	Original	2	0.968	0.566	0.959	0.641	0.942	0.742
		First derivative	7	0.973	0.527	0.955	0.674	0.926	0.842
		Second derivative	2	0.961	0.631	0.949	0.719	0.938	0.769
	PLS	SNV	4	0.930	0.844	0.894	1.035	0.765	1.502
		SG	2	0.968	0.567	0.959	0.642	0.942	0.742
Salbutamol sulfate	PCR	Original	2	0.968	0.566	0.959	0.641	0.942	0.742
		First derivative	2	0.965	0.594	0.955	0.677	0.941	0.753
		Second derivative	2	0.961	0.626	0.949	0.718	0.938	0.771
	PLS	SNV	4	0.934	0.817	0.893	1.043	0.765	1.500
		<b>SG</b>	<b>2</b>	<b>0.968</b>	<b>0.567</b>	<b>0.959</b>	<b>0.642</b>	<b>0.943</b>	<b>0.742</b>

The selected model of calibration for each compound was marked in bold.

PCR: Principal component regression; PLS: Partial least squares; SNV: Standard normal variate; SG: Savitzky–Golay smoothing with polynomial order of 3 and window width of 11 points.

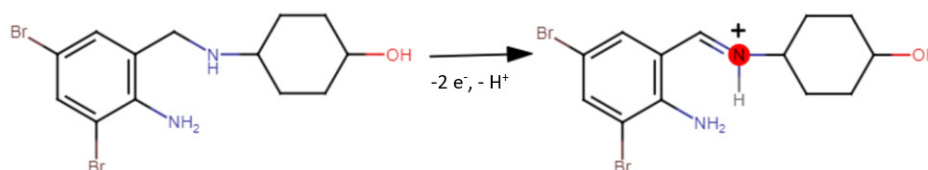
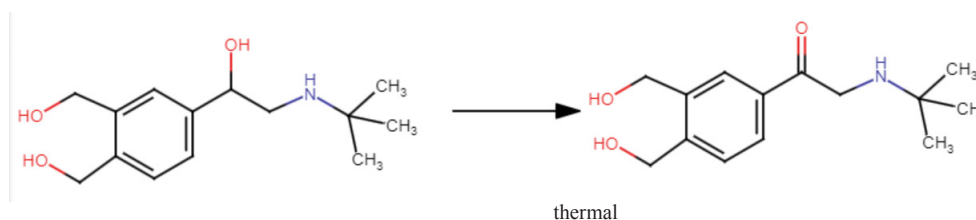


**Figure 4.** Regression coefficient plots for (a) ambroxol HCl and (b) salbutamol sulfate; multivariate regression plots for (c) ambroxol HCl and (d) salbutamol sulfate.

**Table 3.** Data stability of ambroxol HCl and salbutamol sulfate for each storage time.

Analytes	Storage time (days)	Content of analyte (% w/w)
Ambroxol HCl	1	96.99 ± 4.10
	7	103.73 ± 1.53
	14	NA
Salbutamol sulfate	1	99.63 ± 3.66
	7	90.38 ± 1.79
	14	59.68 ± 24.79

NA = Not applicable;  $n = 3$ , and data expressed as mean ± SD.

**Figure 5.** Reaction oxidation of ambroxol (adapted from Levent *et al.*, 2014).**Figure 6.** Reaction oxidation of salbutamol (adapted from Goyal *et al.*, 2007).

salbutamol sulfate using the previous equation for ambroxol HCl and salbutamol sulfate model. The actual value from ambroxol HCl and salbutamol sulfate for each storage time (1, 7, and 14 days) was calculated, and the result was compared to the initial assay. The data are presented in Table 3. Based on the Indonesian Pharmacopoeia edition VI, it is known that the content of the active substance in a solid dosage form for salbutamol sulfate is 90%–100% and for ambroxol HCl it is 85%–105%, based on the value stated on the label. The results of the assay on the preparation on days 1, 7, and 14 (Table 3) showed that there was a change in the levels of ambroxol HCl and salbutamol sulfate. The levels of ambroxol HCl and salbutamol sulfate still met the level requirements until day 7 of storage. On day 14 of storage, the drug content in the preparation was below the specified requirements. From these results, it is known that the DPP samples containing salbutamol sulfate and ambroxol HCl can be stable for up to 7 days with storage conditions at room temperature and 75% RH.

According to the data presented in Table 3, the sample was stable until day 7 in the storage condition because the content was more than 90% of the initial content. It is suitable according to the WHO guidelines of stability testing (2018) which indicated that the potential adverse effect would appear by loss of active ingredient. The degradation of the active pharmaceutical ingredient in the product results in less than 90% of the drug as claimed on

the label or unacceptable quality. Since the therapeutic index of salbutamol sulfate was narrow, it is important to perform further evaluation of quality to minimize the adverse effect. After day 14, the content determination of ambroxol HCl was not successfully performed. It is indicated that the content of salbutamol sulfate was less than 90% after storage in day 14. The decrease in chemical stability from ambroxol HCl and salbutamol sulfate was because both of them were subjected to oxidation and thermal degradation. Ambroxol HCl undergoes degradation under oxidation and heat conditions (Jelić *et al.*, 2021), and salbutamol sulfate was unstable in thermal conditions too (Abdul-jabbar *et al.*, 2021). The reaction degradation of ambroxol HCl and salbutamol sulfate can be seen in Figures 5 and 6.

## CONCLUSION

Evaluation of the DPP samples obtained from a private hospital in Semarang was successfully conducted. Compatibility and stability studies as well as chemometric modeling for content determination were carried out in order to provide comprehensive information on DPP samples. The compatibility data showed that minimum interaction occurred between active pharmaceutical ingredients and excipients from the DPP containing ambroxol HCl and salbutamol sulfate. The chemometrics of multivariate calibration was successfully generated for both ambroxol HCl

and salbutamol sulfate content determination. Since selected multivariate calibration models were obtained to predict the content of the two analytes, here we exploited these models to calculate the actual content value of ambroxol HCl and salbutamol sulfate in the samples. A stability study of 14 days' evaluation was carried out by determining the content of active pharmaceutical ingredients including ambroxol HCl and salbutamol sulfate. It was found that the sample was stable until day 7 in the storage condition.

However, the evaluation of the degradation product was not conducted in this study due to our limitation. Since the specificity of the degradation product should be observed, it is important to develop other analytical techniques such as high performance liquid chromatography and liquid chromatography–mass spectrometry in order to obtain degradation product characteristics according to analytes separation and identification.

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#### CONFLICTS OF INTEREST

All the authors declared there are no conflicts of interest.

#### AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

#### ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

#### DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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