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THE INFLUENCE OF GRINDING TIME AND EQUIPMENT ON THE HOMOGENEITY AND STABILITY OF DIVIDED POWDERS CONTAINING SALBUTAMOL SULPHATE AND AMBROXOL HYDROCHLORIDE

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ABSTRACT

The prevalence of compounding divided powder remains high in pediatric patients due to the limitation of licensed drugs. Divided powder was compounded for easy dosage adjustment. Previous studies showed that the homogeneity of pulveres didn`t meet the requirement and was Beyond the Use Date of less than 30 days. The study aims to enhance the homogeneity and stability quality of divided powder compounding containing salbutamol sulphate and ambroxol hydrochloride. The process was carried out using mortar-pastle and pulverizer with 1, 3, and 5-minute grinding time. Determination of active substances using UV-spectrophotometer with PLSR and PCR chemometrics models with data preprocessing. Evaluation results from the most optimal chemometric model to determine the content. The best model was PCR, with the first derivative for ambroxol hydrochloride and the second derivative for salbutamol sulphate. The result of the compounding process didn`t meet the homogeneity test for 30 samples. The BUD of ambroxol hydrochloride was more than 1 month with a pulverizer and more than 4 months with a mortar pestle. Therefore, good recommendations for a divided powder compounding process can be made with a 1-minute grinding time with a pulverizer or mortar-pestle with 1 or 3 minutes grinding time.

Keywords: Pulverizer, Mortar-Pestle, Homogeneity, Beyond Use Date, Divided Powder Quality, Quality Improvement.

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INTRODUCTION

Over 80% of divided powders, were prescribed for pediatric patients in Indonesia to anticipate the limitation of licensed medication.[1] It contained one or more active pharmaceutical ingredients. The advantages include the ability to dosage adjustments, more stable than liquid dosage form, and easy administration for patients who have difficulty swallowing tablets or capsules. The quality should be ensured for patient safety.[2,3,4] Recent studies show that Beyond the Use Date, this solid dosage form in Indonesian health facilities was less than 20 days, shorter than the United States Pharmacopeia recommendation.[5] Mortar-pestle and pulverizer were equipment to make divided powder. Time grinding and visual division were critical processes that affected the quality. Both equipment generated thermal energy through pressure and friction with drug substances. However, a pulverizer was more beneficial when receiving numerous prescriptions with limited time. The challenge was a risk of significant yield loss and reduced homogeneity. It was important to ensure the quality of extemporaneous preparation for patient safety.[6,7,8] However there was no guidance for time grinding to ensure homogeneity and stability of divided powder. Research needed to be conducted on the effect of grinding time and equipment on the homogeneity and stability of extemporaneous. The study aims to enhance content homogeneity and storage time of divided powder Salbutamol sulphate 2 mg and ambroxol hydrochloride 15 mg were selected based on frequently prescribed combinations which were heat-stable in the compounding process and salt form. It was to overcome cough and asthma in pediatric patients.[9,10] Spectrophotometric-assisted chemometric Partial Least Square Regression and Principal Component Analysis were used to determine the combination of active pharmaceutical combinations without separation.[11,12] The advantage was rapid, sensitive, and cheap.[13]

EXPERIMENTAL

Material and Methods

The working standard of ambroxol hydrochloride and salbutamol sulphate was obtained from PT Ifars Solo, Center of Java, Indonesia. Methanol pro analysis from Smart-Lab. Ambroxol hydrochloride 30 mg tablets

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were obtained from PT. Etercon Pharma Demak, Indonesia. Salbutamol sulphate 4 mg tablets was obtained from PT. Yarindo Farmatama, Serang, Indonesia.

General Procedure

To build multivariate models, a mixture of standard solutions was created from salbutamol sulphate and ambroxol hydrochloride in random concentrations. A divided powder sample combination was compounded from salbutamol sulphate and ambroxol hydrochloride tablets. 180 units of sample were used for the homogeneity test, and 210 units were used for the chemical stability test. The multivariate models were used to determine the sample content for the homogeneity and stability tests.

Developing of Multivariate Model

The working standard powder of salbutamol sulphate weighed 10 mg. Then, it dissolved with methanol pro analysis in a 10 mL volumetric flask to obtain a salbutamol sulphate standard solution of 1000 ppm. The same process was performed for working standard ambroxol hydrochloride to prepare 1000 ppm. Both solutions were pipetted in specific volumes into a 5 mL volumetric flask. Methanol pro analysis was added to the mark to build 30 calibration (C) and 15 validation (V) sets.[14] The ambroxol hydrochloride (AH) range was 20-45 ppm while salbutamol sulphate (SS) was 3-10 ppm in Table-1.

Divided Powder Sample Compounding

Divided powder in this study consisted active substance combination of salbutamol sulphate 2 mg and ambroxol hydrochloride 15 mg.[10] The sample compounding was performed in the Faculty of Pharmacy Sanata Dharma University, Yogyakarta, Indonesia. The samples were prepared by the following prescription:

R/ Ambroxol hydrochloride 30 mg ½ tab
Salbutamol sulphate 4 mg ½ tab
M f pull dtd no X

For the homogeneity test: 5 tablets of ambroxol hydrochloride 30 mg and 5 tablets of salbutamol 4 mg sulphate were put into a pulverizer. They were ground for 1 minute and divided into 10 equal units using an analytical balance. This process was repeated three times to obtain 30 samples. Then, the same procedure was followed for 3 and 5 minutes of grinding time. The sampling process was applied using mortar-pestle with geometric dilution techniques for 1, 3, and 5 grinding times.[15] The packaging used a divided powder punch and then placed into plastic clip bags, then silica gel was added. For the stability test: 5 tablets of ambroxol hydrochloride 30 mg were put into a mortar pestle. Then tables were grinded for 1 minute and divided into 10 equal units with an analytical balance. The same process was done for salbutamol sulphate 4 mg. Salbutamol powder was divided into 10 equal units in the same packaging as ambroxol and put into the plastic clip with gel silica. This process was repeated to obtain 35 samples for one-time treatment. The process was also done for 3 and 5-minute grinding time. After that, samples were stored in a climatic chamber with 30 ºC and 75% relative humidity.[16] Each 5 samples were analyzed at day-0, 7, 14, 21, 28, 60, and 90 to obtain active content. The same process was repeated for pulverizer equipment.

Table-1: The Concentration of calibration and Validation Set for Ambroxol and Salbutamol

Number	Calibration set concentration (ppm)		Number	Calibration set concentration (ppm)		Number	Validation set concentration (ppm)	
	AH	SS		AH	SS		AH	SS
C1	30	7	C16	28	4	V1	29	6
C2	31	3	C17	25	6	V2	41	3
C3	26	6	C18	40	5	V3	28	8
C4	39	3	C19	35	3	V4	35	5
C5	29	5	C20	37	6	V5	27	8
C6	44	7	C21	31	10	V6	30	3
C7	38	3	C22	45	3	V7	36	3
C8	36	4	C23	44	5	V8	26	4
C9	37	8	C24	28	7	V9	25	6
C10	42	7	C25	27	6	V10	27	3

C11	33	3	C26	20	5	V11	45	10
C12	44	4	C27	23	6	V12	20	9
C13	34	7	C28	22	3	V13	23	7
C14	38	3	C29	24	6	V14	22	10
C15	36	9	C30	21	4	V15	25	4

C: calibration, V: validation, AH: ambroxol hydrochloride, SS: salbutamol sulphate, ppm: part per million.

Sample Preparation

Divided powder samples were dissolved with methanol pro analysis solvent into a 5 mL volumetric flask. Later, they were filtered with filter paper. The filtrate was pipetted 50 µL into a 5 mL new volumetric flask. This solvent was added to the mark to make a sample solution.

Detection Method

The calibration, validation set, and sample solution were scanned in a UV-spectrophotometer double-beam at 240-350 wavelengths range with 2 nm intervals.[10,17] The output was an original spectrum consisting of ambroxol and salbutamol absorbance. The calibration and validation set were used to predict the sample content. This was used to determine homogeneity and stability tests.

Data Analysis

The data analysis used *Rstudio*® software version 2023.6.1.524. The original spectrum was transformed into Standard Normal Variate, Multiplicative Scatter Correction, Savitsky-Golay, First, and Second Derivative to obtain the good-fit model. Their spectrums were evaluated in 3 parts. It consisted of a calibration set, internal validity leave-one-out from the calibration set, and external validity from the validation set.[18,19,20] The god-fit model was chosen by the highest accuracy model (R^2) and the lowest error model. The R^2 was determined from the determination coefficient between actual and prediction values.[21] It included R_{cal}^2, R_{CV}^2, and R_{Val}^2. The error model was determined from Root Mean Square Error consisting of RMSEC, RMSECV, and RMSEP. [22,23] The homogeneity test was carried out with a standard deviation (SD), mean, and coefficient of variation (CV), for 10, 20, and 30 sample units at each treatment. The test met the requirements of the CV value wasn`t more than 5%.[14] The stability test was carried out with a correlation between active content (c), Ln(c), and 1/(c) versus days of testing. After that, intercept, slope, and correlation coefficient (R) were calculated. The good value of the correlation coefficient selected reaction order. The BUD was calculated with the t_{90} formula.[22]

RESULTS AND DISCUSSION

Multivariate Analysis and Selecting Model

Before creating a multivariate model, a qualitative test was conducted to observe spectra profiles of single and mixture analytes based on prescription. Scanning was made from a standard solution. The ratio of salbutamol sulphate and ambroxol hydrochloride was 1:7.5. The solution concentration was the same as the proportion of sample preparation.

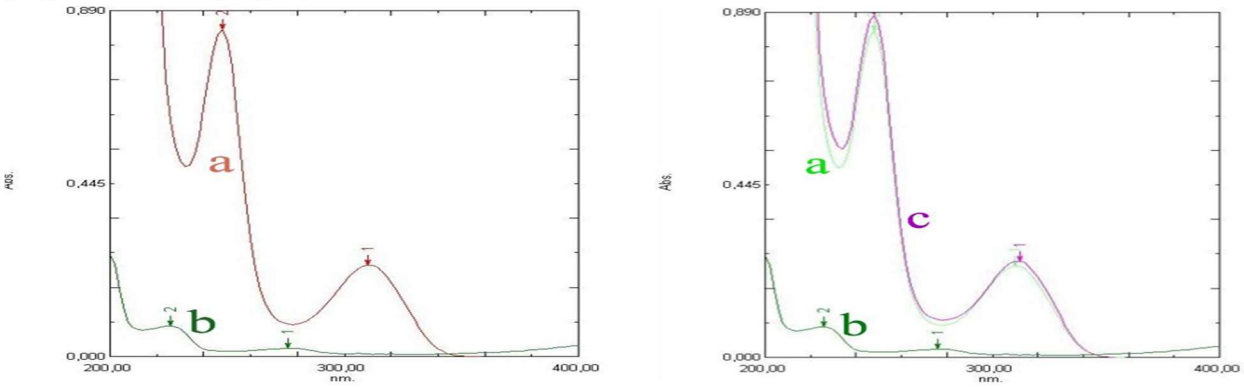

Fig.-1: The Spectrum Ratio Standard Solution of Salbutamol Sulphate and Ambroxol Hydrochloride Based on Prescription. A: Standard Solution of Ambroxol Hydrochloride 30 ppm, B: Standard Solution of Salbutamol Sulphate 4 ppm, C: Mixture from Ambroxol Hydrochloride 30 ppm and Salbutamol Sulphate 4 ppm

Figure-1 shows the overlapping between salbutamol sulphate 4 ppm and ambroxol hydrochloride 30 ppm. However, the mixed spectrum was identical to the single spectrum of ambroxol hydrochloride. Figure-2 shows the overlapping of calibration and validation standard solutions to build multivariate models.

The spectrophotometric method had limitations in determining mixture analytes simultaneously. The chemometric technique with PLSR and PCR provides a solution to overcome this problem. The spectrum range 240-350 nm with a 2 nm interval was chosen for analysis due to absorbance contribution from analytes. This original spectrum was converted to MSC, SNV, Savitzky-Golay, First, and Second Derivative. This preprocessing data could improve the good-model result of the multivariate model. The evaluation for the calibration data set included R_{cal}^2 and RMSEC, and then internal validity with the Leave-One-Out technique was calculated to obtain R_{CV}^2 and RMSECV. The validation data set was used to determine R_{val}^2 and RMSEP. Both internal and external validity could anticipate an overfitting model in a calibration set. The number of components was determined by the lowest value of RMSECV in internal validity.

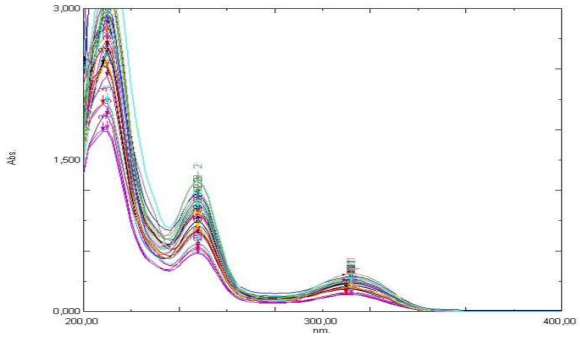

Fig.-2: Overlapping of Mixture Standard Solution for Calibration and VALIDATION SET. They were created from Salbutamol Sulphate and Ambroxol Hydrochloride with Random Concentrations

Based on Table-2, the model was selected from the Principal Component Regression chemometric technique. For salbutamol sulphate, the spectrum was chosen from the Second Derivative with values of R_{cal}^2: 0.998; RMSEC: 0.090; R_{CV}^2: 0.924; RMSECV: 0.609; R_{Val}^2: 0.896; and RMSEP: 0.893. For ambroxol hydrochloride was picked up from value of R_{cal}^2: 0.857; RMSEC: 2.787; R_{CV}^2: 0.841; RMSECV: 2.933; R_{Val}^2: 0.979; and RMSEP: 0.989. This selection was based on R^2 close to 1 and RMSE close to 0. The selection model is used to determine the analyte content. Then, it was used to calculate homogeneity and stability tests.

Table-2: Multivariate Analysis Evaluation with PLSR and PCR Techniques for all Spectra

Analytes	Techniques	Type of Spectra	Number of Components	Calibration Set		Internal Validity		External Validity	
				R_{cal}^2	RMSEC	R_{CV}^2	RMSECV	R_{Val}^2	RMSEP
Ambroxol Hydrochloride	**PCR**	OR	1	0.842	2.929	0.826	3.065	0.976	1.065
		MSC	18	0.920	2.082	0.545	4.962	0.553	4.570
		SNV	23	0.957	1.522	0.499	5.211	0.610	4.268
		SG	1	0.838	2.959	0.823	3.094	0.974	1.102
		1st der	**1**	**0.857**	**2.787**	**0.841**	**2.933**	**0.979**	**0.989**
		2nd der	1	0.857	2.786	0.841	2.928	0.978	1.012
	PLSR	OR	1	0.842	2.926	0.827	3.065	0.976	1.062
		MSC	8	0.968	1.318	0.491	5.252	0.607	4.286
		SNV	8	0.967	1.329	0.420	5.604	0.640	4.100
		SG	1	0.839	2.954	0.823	3.094	0.974	1.095
		1st der	1	0.857	2.787	0.841	2.935	0.979	0.889
		2nd der	1	0.857	2.780	0.841	2.932	0.978	1.013

Salbutamol Sulphate	**PCR**	OR	27	0.999	0.073	0.907	0.674	0.931	0.726
		MSC	26	0.997	0.125	0.833	0.904	0.815	1.190
		SNV	26	0.997	0.114	0.818	0.945	0.825	1.158
		SG	25	0.999	0.082	0.899	0.704	0.943	0.659
		1st der	28	1.000	0.006	0.926	0.601	0.923	0.768
		2nd der	**26**	**0.998**	**0.090**	**0.924**	**0.609**	**0.896**	**0.893**
	PLSR	OR	11	0.999	0.073	0.903	0.689	0.931	0.729
		MSC	7	0.981	0.302	0.838	0.890	0.839	1.109
		SNV	8	0.991	0.207	0.830	0.913	0.845	1.091
		SG	16	0.999	0.060	0.898	0.708	0.935	0.705
		1st der	11	1.000	0.031	0.928	0.592	0.921	0.780
		2nd der	9	0.999	0.076	0.916	0.641	0.899	0.881

Bold mark: selected model for each analyte; PLSR: Partial Least Square Regression, PCR: Principal Component Regression; OR: original spectra; MSC: Multiplicative Scatter Correction, SNV: Standard Normal Variate; SG: Savitzky-Golay; 1st der: First Derivative; 2nd der: Second Derivative.

Result of Homogeneity Test from Difference Treatments

The determination of the homogeneity test is based on the percent coefficient of variance from 10-, 20-, and 30-unit samples at each treatment. Table-3 shows the homogeneity test result of active substance for salbutamol sulphate and ambroxol hydrochloride analytes. The divided powder was compounded by mortar-pestle versus pulverizer with 1, 3, and 5-minute grinding time.

Table-3: The Results of Homogeneity Test Based on Different Equipment and Time-Grinding

Analytes	Number of Treatments	Treatments		% CV		
		Types of equipment	Time grinding	s10	s20	s30
Ambroxol Hydrochloride	1	Mortar-pestle	1 minute	**2.42**	6.44	6.69
	2		3 minutes	7.02	**4.98**	6.73
	3		5 minutes	**3.45**	6.58	5.71
	4	Pulverizer	1 minute	**3.68**	**4.76**	5.42
	5		3 minutes	**3.7**	**4.74**	5.41
	6		5 minutes	**3.08**	6.25	5.53
Salbutamol Sulphate	1	Mortar-pestle	1 minute	7.24	25.08	20.56
	2		3 minutes	29.4	39.79	37.36
	3		5 minutes	26.01	25.68	25.1
	4	Pulverizer	1 minute	33.71	30.33	35.06
	5		3 minutes	25.84	28.18	27.77
	6		5 minutes	36.11	28.18	25.17

Bold mark: %CV met the homogeneity test requirement, s10: sample from 10 divided powder, 20: sample from 20 divided powder, s30: sample from 30 divided powder.

Eight results from 10 and 20 samples for ambroxol hydrochloride meet the homogeneity requirement due to %CV less than 5%. However, another result from 30 samples was close to the homogeneity requirement. For salbutamol, sulphate did not meet the homogeneity test due to the high CV value and smaller ratio than ambroxol hydrochloride. At each treatment, the sample was compounded 10 units three times to obtain 30 samples. It same with prescriptions and anticipate the high variation at unit dose. In Fig.-3, the amount of 10 units of samples for ambroxol hydrochloride met the homogeneity criteria except for mortar-pestle with 3 minutes of grinding time. Compounding divided powder with a pulverizer had the same homogeneity as mortar-pestle. It was shown at treatments 1 and 3. Compounding with a pulverizer gave less energy and time for tablet grinding compared with mortar-pestle. However, it required 10 tablets for minimum grinding. The homogeneity of divided powder had an impact on decreasing the variation of content. It would increase drug safety for patients who have difficulty with tablets or capsule swallowing.

Result of Stability Test from Divided Powder

The stability test was used to determine the BUD of ambroxol hydrochloride and salbutamol sulphate for 90 days. Table-4 shows the impact of equipment and time grinding on extemporaneous storage time.

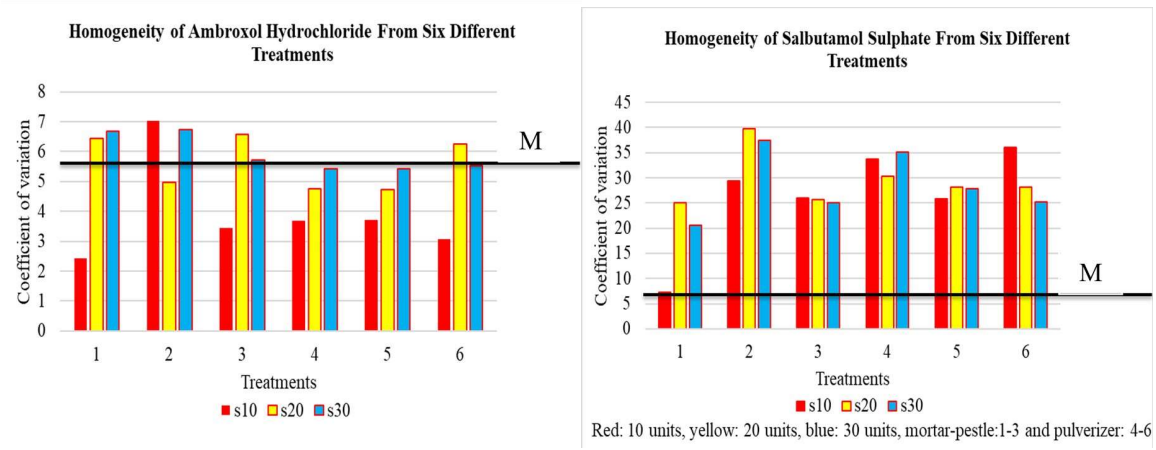

Fig.-3: Comparison of the Homogeneity Test Between Ambroxol Hydrochloride and Salbutamol Sulphate. M: Maximum Homogeneity Requirement ≤ 5%

The kinetic reaction order for ambroxol hydrochloride followed the first order while salbutamol sulphate for zero and first order. The selection of reaction order is based on the highest correlation coefficient between time vs (c), time vs Ln(c), and time vs 1/(c). It referred to zero, one, and second order. However, the stability test of divided powder was suitable for ambroxol hydrochloride due to good coefficient correlation.

Table-4: The Result of the Stability Test Based on Different Equipment and Time-Grinding

Analytes	Number of treatment	Treatments		Reaction order	Slope	R	BUD (days)
		Types of Equipment	Time grinding				
Ambroxol Hydrochloride	1	Mortar-pestle	1 minute	1	-0.0008	-0.811	133.3
	2		3 minutes	1	-0.0008	-0.984	128.5
	3		5 minutes	1	-0.0021	-0.838	49.7
	4	Pulverizer	1 minute	1	-0.0023	-0.933	45.9
	5		3 minutes	1	-0.0048	-0.970	21.8
	6		5 minutes	1	-0.0055	-0.927	19.1
Salbutamol Sulphate	1	Mortar-pestle	1 minute	1	-0.0070	-0.674	15
	2		3 minutes	1	-0.024	0.002	64.2
	3		5 minutes	1	-0.0050	-0.529	20.9
	4	Pulverizer	1 minute	0	-0.3334	-0.129	38.7
	5		3 minutes	1	-0.0061	-0.566	17.2
	6		5 minutes	1	-0.7208	-0.011	9.6

R: correlation coefficient, BUD: Beyond Use Date

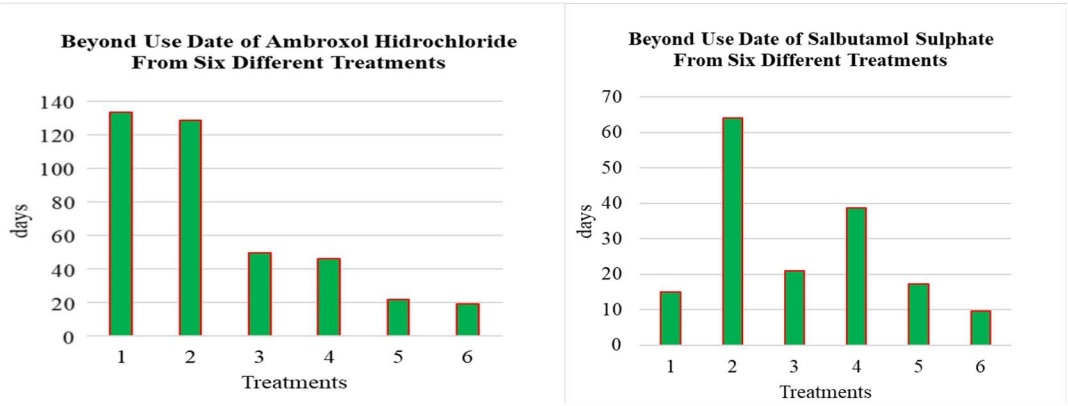

Fig.-4: Comparison of Beyond Use Date between Ambroxol Hydrochloride and Salbutamol Sulphate

The result of Beyond Use Date divided powder with mortar-pestle showed longer BUD than pulverizer. In the pulverizer, the friction between the blade and tablets would decrease active substance, reducing BUD. Increasing of time grinding shows inversely proportional due to increasing kinetic energy. This is shown in Fig.-4. Thus, the active substances were degraded. The recommended compounding process by mortar-pestle was 1 and 3 minutes while the pulverizer was 1 minute. Ambroxol hydrochloride with mortar-pestle equipment with time grinding of 1 and 3 minutes gave BUD more than 4 months, while the pulverizer was 1 month. The 3-month duration for time stability gave longer BUD than the previous study. However, the result was still far from the USP recommendation. Both treatments would provide more than 45 days of storage time at room temperature with 30 ºC and 75% relative humidity.

CONCLUSION

The best chemometric model for determining ambroxol hydrochloride and salbutamol sulphate was PCR with first and second derivative spectra. The compounding process from the pulverizer and mortar-pestle with 1, 3, and 5 grinding times gave good homogeneity for ambroxol hydrochloride. It was for 10 and 20 samples. Compounding with mortar-pestle for 1- and 3-minute grinding time provided BUD more than 4 months while 1 minute gives 45 days for ambroxol hydrochloride. Therefore, to ensure the good homogeneity and stability quality of divided powder can be done with a 1-minute pulverizer or 1 or 3 minutes grinding time.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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