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Original Research Article

Development and Validation of Purple Sweet Potato (*Ipomea batatas L.*) Pigment as a Titrimetric Indicator for Hydrochloric Acid Quantification

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ABSTRACT

An analytical method development requires a validation procedure. The method is valid if it meets certain performance criteria. The purpose of this study was to validate purple sweet potato (PSP) pigment (PSPP) as titration indicator (TI) for hydrochloric acid quantification. PSPP was extracted using analytical-grade (AG) and medical-grade (MG) ethanol. PSPP extracted by AG and MG ethanol were designated PSPP-AG and PSPP-MG, respectively. PSPP-AG and PSPP-MG were used as indicators in the titration of HCl with NaOH, and the method was validated by assessing the following parameters; precision, accuracy, linearity, limit of quantification (LoQ), limit of detection (LoD), robustness, and uncertainty. Results demonstrated that PSPP-AG and PSPP-MG have high linearity with R^2 values of 0.9990 and 0.9991, respectively. They also have high accuracy with recovery of 102.4%, and high precision with coefficient of variation (CV) ranging from 0.1609% to 0.8773%. PSPP-AG and PSPP-MG gave LoD of 0.0220 M and 0.0213 M, and LoQ of 0.0734 M and 0.0709 M, respectively. PSPP-AG and PSPP-MG had excellent reproducibility, with CV ranging from 0.199% to 0.518%, and low uncertainty (0.000262 M and 0.000905 M). PSPP-AG and PSPP-MG are robust indicators; they were insensitive to changes in treatment conditions, with t-stat < t-table of 3.475 < 4.303 for variations in sample volume, and 1.380 < 12.706 for variations in NaOH concentration. The HCl content quantified by PSPP-AG and PSPP-MG was 0.041 M, the same as that obtained with methyl red and phenolphthalein. Therefore, PSPP-MG and PSPP-AG are valid indicators for HCl quantification in pharmaceutical dosage forms.

Keyword: Validation, Method development, Accuracy, Precision, Natural pigment.

Introduction

Titrimetric analysis is a conventional quantitative analysis method that is still being used in routine analysis till date. The application of this method has greatly contributed to the advancement of the chemical industry and chemical science.[1] Titrimetric analysis is simple, low-cost, and readily available in teaching and research laboratories.[1,2] Titrimetric is an important topic and must be studied at high school and college levels.[3–5] In this method, an indicator is needed to detect the end point. Methyl red, phenolphthalein, and bromothymol blue are the standard laboratory indicators mostly used in neutralization titrations, but they are costly, hazardous, and environmentally harmful.[6] The use of these indicators in routine analysis for teaching and research does not support green chemistry,[7–9] hence the need for eco-friendly natural plant-based pigments like PSPP.[10]

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PSP tubers are one of Indonesia's agricultural products, which are rich in anthocyanins.[11–15] Anthocyanins possess special biological properties, including antioxidant, anti-inflammatory,[16] antidiabetic,[17] and antimicrobial properties.[18] They also exhibit unique colors in different pH environments;[19,20] dark red (pH 1), pink (pH 2-6), purple (pH 7), blue (pH 8-9), green (pH 10-11), and yellow (pH 12-14).[21–23] This property makes it possible for PSPP to be applied as a titration indicator, but its application has been limited to titration of HCl with NH_4OH and HCl with NaOH. The most recent progress in the use of PSPP was reported by Leba *et al*, where they explored PSPP for acid determination in pharmaceutical dosage forms containing HCl, but they only studied its stability, precision, accuracy, and performance for acid determination.[21] Their study showed that PSPP has excellent performance comparable to phenolphthalein, but no report on the validation of the method was provided. Thus, there is the need for a more in-depth study on the validation of PSPP as a TI. The novelty of this study is to develop and validate the use of PSPP as a TI for acid determination in pharmaceutical dosage forms containing HCl.

In routine analysis, an analytical method can be used if the method is appropriate, and has been validated.[2] There are several method validation parameters, they include linearity, LoD, LoQ, precision, selectivity, accuracy, robustness, specificity, ruggedness, and uncertainty.[24–26] The development of PSPP as an indicator must also meet these criteria. By meeting the validation criteria, PSPP might be proposed as an indicator for titrimetric analysis. The purpose of this study was to develop and validate the use of PSPP as a TI for the quantification of hydrochloric acid in pharmaceutical dosage form and compare with standard indicators (phenolphthalein, and methyl red).

Materials and Methods

Chemicals
Analytical-grade ethanol (96%, Merk, Indonesia), medical-grade ethanol (95%, One Med, Indonesia), hydrochloric acid (37%, Merk, Indonesia), sodium hydroxide (99%, Merk, Indonesia), oxalic acid (Merk, Indonesia), distilled water were the chemicals used in this work.

Plant material collection and preparation
PSP tubers were purchased on 11th May 2024 at the local market in Kupang City, Indonesia (10°08'34.8"S 123°39'12.7"E). The PSP was cleaned, washed, sliced, dried, and ground into flour.[21]

Extraction of purple sweet potato pigment (PSPP)
The extraction of PSPP was carried out in two steps. Step 1: PSP flour was macerated in MG ethanol (1:3 g/mL).[21] Step 2: PSP flour was macerated in AG ethanol (1:3 g/mL). The extractions were carried out in an acidic environment for 24 hours. The extracts were concentrated to half their initial volume. The pigment obtained from the first extraction step was designated PSPP-MG, while that obtained from the second step was designated PSPP-AG.[21] PSPP-MG and PSPP-AG were the proposed indicators used in this study.

Standardization of sodium hydroxide (NaOH) solution
The NaOH solution used in this work was standardized with 0.10 M oxalic acid ($H_2C_2O_4.2H_2O$).

Validation of PSPP as a TI
The titrimetric method based on the use of PSPP as TI was validated by assessing the performance of the method based on the following parameters; linearity, LoD, LoQ, precision (repeatability), accuracy, robustness, reproducibility, and uncertainty.

Determination of linearity
The linearity of proposed method was determined by the titration of a series of HCl solutions (0.025 M, 0.05 M, 0.15 M, 0.20 M, 0.25 M, and 0.30 M) with NaOH (0.102 M) using the proposed indicators (PSPP-MG and PSPP-AG).[2,27] A number of 10 mL of each HCl was titrated with 0.102 M NaOH. The titration was stopped after the indicator changed color from red to blue. The titrations were carried out seven times. The titer values (volume of NaOH used) obtained were used to prepare a standard calibration curve for the relationship between NaOH volume and HCl concentration. From the curve, the regression equation and correlation coefficient (R^2) were obtained. The regression equation is an expression of the linear relationship between the instrument response (y) and the standard concentration (x) over a certain concentration range (equation 1).[28] In this study, the regression equation expresses the linear relationship between the volume of NaOH standard solution (y) and the concentration of HCl (x). According to the Association of Official Analytical Chemists (AOAC), the linearity of a method is declared good if the R^2 value obtained is more than 0.990.[29]

$$y = bx + a \quad \text{..... 1}$$

Where;
y is the response of indicator in terms of volume of NaOH, *x* is the concentration of HCl, *a* is the intercept of the calibration curve, *b* is the slope of the linear regression equation of calibration curve.

Determination of limit of detection (LoD)

The LoD was determined from the regression equation of the calibration curve according to the formula shown in equation 2.[28]

$$LoD = 3\,(S_a/b) \quad \text{..... 2}$$

Determination of limit of quantification (LoQ)

The LoQ was determined from the regression equation of the calibration curve according to the formula in equation 3.[24,28]

$$LoQ = 10\,(S_a/b) \quad \text{..... 3}$$

Where
b is the slope of the regression equation of calibration curve, and S_a is the standard deviation of the response. S_a can be estimated by the standard deviation of y-residual or y-intercept.[28] In this study, S_a was estimated by the standard deviation of y-intercept (Equation 4).

$$S_a = \text{Standard deviation of intercept}/\sqrt{n} \quad \text{..... 4}$$

Where;
n is the number of observations.

Determination of accuracy
The titration was carried out for samples without spiking (SWoS) and samples spiked (SWS).[21] For SWoS, a single paratusin tablet was broken up, dissolved in distilled water, filtered, and then diluted in a volumetric flask (100 mL). For SWS, a single paratusin tablet was broken up, dissolved in distilled water, filtered, then transferred to a volumetric flask (100 mL), and 1 mL of concentrated HCl was added and diluted to a volume of 100 mL.[21] A total of 10 mL of each sample was titrated with 0.102 M NaOH using the proposed indicators (PSPP-MG and PSPP-AG). The titration was carried out seven times. The accuracy of the proposed indicators was assessed based on the recovery, R (%) of the analyte by titration (equation 5). According to AOAC, a method is declared accurate if the recovery, R (%), is 90%-108%.[21]

$$\text{R}(\%) = \frac{[\text{ASWS}]-[\text{ASWoS}]}{[\text{s}]} \text{ x } 100 \quad \text{..... 5}$$

Where;
[ASWS] : analyte concentration in the spiked sample (M)
[ASWoS] : analyte concentration in the sample without spiking (M)
[s]: concentration of the substance used for spiking (M)

Determination of precision (*repeatability*)
The precision of proposed indicators was determined by carrying out the titration repeatedly. Precision of proposed indicators was expressed as the coefficient of variation, or CV, as shown in equation 6. According to the AOAC, a method is declared precise if the coefficient of variation obtained does not exceed 2%.[21]

$$\text{CV}\ (\%)\frac{\text{SD}}{\bar{\text{x}}}.100\% \quad \text{..... 6}$$

Where;
CV is the coefficient of variation (%)
SD is the standard deviation of repeated titrations
$\bar{x}$ is the average of titrant volume (mL)

Determination of reproducibility
Reproducibility of the proposed indicator was determined by repeating the same titration procedure for three consecutive days.[27] A number of 10 mL of sample was titrated with 0.102 M NaOH. The titration was carried out seven times using the proposed indicators. The proposed indicators is said to have good intra-laboratory reproducibility if the CV value does not exceed 4%.[27]

Determination of robustness
The robustness of the proposed indicator was determined by varying the volume of samples and the concentration of NaOH.[27]
Variation in sample volume: a single paratusin tablet was broken up, dissolved in distilled water, filtered, then moved to a volumetric flask (100 mL), and then diluted to a volume of 100 mL. A 10 mL sample solution was titrated with 0.102 M NaOH using the proposed indicators. The titration was carried out in five replicates. This procedure was carried out for 20 mL and 30 mL samples.
Variation in NaOH concentration: the concentrations of the NaOH used were 0.050 M and 0.102 M. A 10 mL sample solution was titrated with 0.050 M NaOH solution using the proposed indicators. The titration was carried out in five replicates. This procedure was carried out for 0.102 M NaOH.
This method is said to be robust if the results of the t-test obtained are not significantly different.

Determination of uncertainty
The uncertainty of the proposed indicator was determined based on the uncertainty of reproducibility (U_R) and uncertainty of repeatability (U_r), which were expressed according to equations 7 and 8.[27]

$$U_r = t_{95:9} . SD_r \quad \text{..... 7}$$

$$U_R = t_{95:9} . SD_R \quad \text{..... 8}$$

Where;
SD_r is the standard deviation of repeatability
SD_R is the standard deviation of intra-laboratory reproducibility
$t_{95:9}$ is the student t-factor for 9 degrees of freedom and 95% confidence level (2.262)

Determination of HCl content of the sample
A 10 mL of sample solution was titrated with 0.102 M NaOH using PSPP-MG indicator. The titration was carried out in seven replicates. This procedure was utilized for PSPP-AG as indicator.

Determination of HCl content using standard indicators
The same procedure for the determination HCl content of the sample was followed using phenolphthalein, and methyl red as indicators.

Statistical Analysis
All data obtained were described in terms of mean, standard deviation (SD), coefficient of variation (CV), and recovery (R). Linear regression analysis of standard calibration curves was carried out using Microsoft office Excel 2016. LoD and LoQ were determined based on linear regression of the calibration curve. Mean data were subjected to paired sample t-test.

Results and Discussion

Linearity
Linearity test showed a linear relationship between the indicator response (PSPP-AG and PSPP-MG) and concentration of HCl over a certain concentration range. From this relationship, the linear regression and correlation coefficient (R^2) were obtained. The linear regression equations for PSPP-AG and PSPP-MG were y = 98.316x + 0.0603 and y = 98.285x + 0.0604, respectively. The letter "y" represents the indicator's response, and "x" represents HCl concentration (M). The indicator response at the end point of the titration correlates with the volume of NaOH; thus, it was expressed in terms of the volume of NaOH. The R^2 values for PSPP-AG and PSPP-MG were 0.9990 and 0.9991, respectively (Figure 1 and Table 1). Ideally, R^2 value should be close to one or higher than 0.995,[29,30] but often values higher than 0.990 are acceptable and adequate.[29] The R^2 value obtained in this study demonstrated that there was a very strong relationship between the volume of NaOH and the HCl concentration. This means that PSPP-AG and PSPP-MG produced a very clear color shift at the end point of the titration. These data demonstrate that PSPP-AG and PSPP-MG show high linearity for HCl quantification.

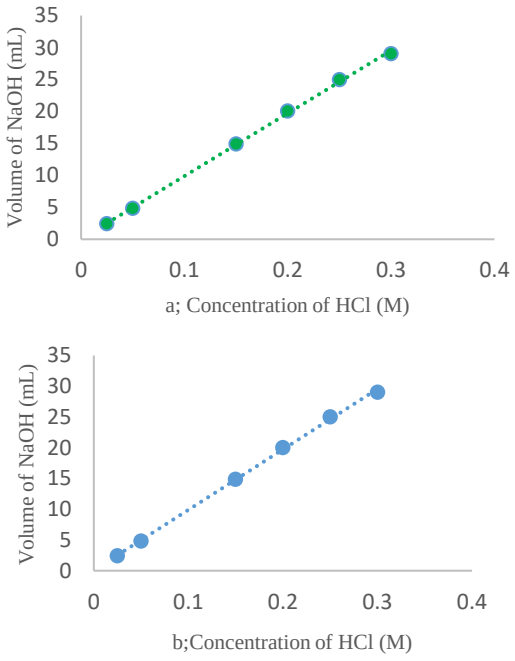

Figure 1: Linearity of proposed indicators **(a)** PSPP-MG, **(b)** PSPP-AG

Limit of detection (LoD) and limit of quantification (LoQ)
The LoD value is the lowest analyte concentration in the sample that can be detected but not measured quantitatively.[2] LoQ represents the lowest analyte concentration in the sample that can be measured with the highest accuracy and precision.[2] The LoD and LoQ are summarized in Table 1. The LoD of PSPP-MG and PSPP-AG were 0.0213 M and 0.0220 M, respectively, and the LoQ were 0.0709 M and 0.0734 M for PSPP-MG and PSPP-AG, respectively.

Accuracy and precision
The accuracy of the proposed indicators was analyzed based on the recovery (%) of HCl. As shown in Table 2, the recovery of HCl using the proposed indicators (PSPP-MG and PSPP-AG) was 102.44%, while the recovery with phenolphthalein indicator was 102.48%. According to AOAC, a method is declared accurate if the recovery is 90%-108%.[21,27] The results of this study show that the proposed indicators have high accuracy, which was comparable to that of phenolphthalein. Precision was assessed based on titration repeatability, and expressed as a coefficient of variation (CV%). Titrations were carried out with spiked samples and with samples without spiking using the proposed indicators, and the results are presented in Table 2. The CV value of HCl concentration for all titrations ranged from 0.1609% to 0.8773%. According to AOAC, a method is declared to have good precision if the CV obtained does not exceed 2%.[21,27] This indicate that the proposed indicators have good precision.

Reproducibility
Reproducibility is the ability to obtain similar results when an experiment is repeated. The reproducibility carried out in this study was intra-laboratory reproducibility. This was done by analyzing samples using the proposed indicators on three different days. The results obtained are displayed in Table 3. The results showed that the CV values obtained were acceptable (0.199% to 0.518%). The value was lower than 4%, which indicates that the proposed indicators have excellent reproducibility.[27]

Robustness
A method that is relatively insensitive to changes in experimental conditions (temperature, acidity, time, etc.) is considered robust.[31] The robustness of the proposed indicators was evaluated by varying the sample volume and NaOH concentration. The results obtained are displayed in Table 4. For variations in sample volume, the t-stat < t-table obtained was 3.475 < 4.303 for PSPP-AG and PSPP-MG. This means that there was no significant difference in the HCl concentration obtained from this treatment. For variations in NaOH concentration, the t-stat < t-table obtained was 1.380 < 12.706 for PSPP-AG and PSPP-MG. This also means that there was no significant difference in the HCl concentration obtained from this treatment. These data indicate that treatment variations do not affect the performance of PSPP-AG and PSPP-MG as indicators. Thus, PSPP-AG and PSPP-MG are said to be robust indicators.

Uncertainty
The uncertainty of the proposed indicators was assessed based on the uncertainty of repeatability (Ur) and reproducibility (UR), as shown in Table 5. These results indicate that the uncertainty of the proposed indicators for quantification of HCl is very small, ranging from 0.000262 to 0.000905 M.

HCl content of the sample
The HCl content of the sample titrated with standard NaOH was calculated using the linear regression equation (Table 1). From this calculation, the HCl content determined using PSPP-AG and PSPP-MG were 0.0404 M and 0.0403 M, respectively (Table 6). The HCl content obtained fell on the linear regression line of the calibration curve.

Table 1: LoD, and LoQ of the proposed indicators

Regression Statistics	PSPP-MG	PSPP-AG
Linear range (M)	0.025-0.30	0.025-0.30
Linear regression equation	y = 98.285x + 0.0604	y = 98.316x + 0.0603
Correlation coefficient (R^2)	0.999080081	0.999012121
Slope of the calibration curve (b)	98.285	98.316
Standard error of intercept	0.284320564	0.294739773
Number of observations	6	6
Standard deviation of intercept (S_a)	0.696440305	0.721962051
LoD (M)	0.02125778	0.022029844
LoQ (M)	0.070859267	0.073432814

Table 2: The accuracy and precision of the proposed indicators

Titration	Spike [HCl]	[HCl] of sample without spike		[HCl] of sample with spike		Recovery of [HCl] (%)	
		PSPP-MG	PSPP-AG	PSPP-MG	PSPP-AG	PSPP-MG	PSPPAG
1	0.1206	0.0421	0.0421	0.1658	0.1658	102.5705	102.5705
2	0.1206	0.0417	0.0412	0.1653	0.1648	102.4876	102.4876
3	0.1206	0.0412	0.0417	0.1648	0.1653	102.4876	102.4876
4	0.1206	0.0412	0.0417	0.1648	0.1648	102.4876	102.0730
5	0.1206	0.0412	0.0412	0.1648	0.1648	102.4876	102.4876
6	0.1206	0.0417	0.0412	0.1648	0.1648	102.0730	102.4876
7	0.1206	0.0412	0.0412	0.1648	0.1648	102.4876	102.4876
Average		0.0415	0.0415	0.1650	0.1650	102.4402	102.4402
SD		0.0004	0.0004	0.0004	0.0004	0.1648	0.1648
CV (%)		0.8773	0.8773	0.2384	0.2384	0.1609	0.1609

[HCl]: concentration of HCl in molarity (M), SD: standard deviation, CV (%): coefficient of variation

Table 3: The reproducibility of the proposed indicators

Day	NaOH ± SD (mL)[a]		[HCl] ± SD[b]	
	PSPP-MG	PSPP-AG	PSPP-MG	PSPP-AG
1	4.071 ± 0.027	4.050 ± 0.029	0.0415 ± 0.0003	0.0413 ± 0.0003
2	4.057 ± 0.035	4.029 ± 0.027	0.0414 ± 0.0004	0.0411 ± 0.0003
3	4.071 ± 0.027	4.071 ± 0.027	0.0415 ± 0.0003	0.0415 ± 0.0003
Average	4.066	4.050	0.0415	0.0413
SD	0.008	0.021	0.0001	0.0002
CV (%)	0.199	0.518	0.2412	0.4843

[a]Mean value of NaOH ± SD from seven replications, [b]Mean value of [HCl] ± SD from seven replications, [HCl]: concentration of HCl in molarity (M), SD: standard deviation, CV (%): coefficient of variation

Table 4: The robustness of the proposed indicators

Variation of treatment		[HCl] ± SD[c]		t-statistic		t-table	
		PSPP-MG	PSPP-AG	PSPP-MG	PSPP-AG	PSPP-MG	PSPP-AG
Volume of sample (mL)	10	0.0413 ± 0.0005	0.0414 ± 0.0004	3.457	3.457	4.303	4.303
	20	0.0405 ± 0.0003	0.0407 ± 0.0002				
	30	0.0407 ± 0.0002	0.0407 ± 0.0002				
Concentration of NaOH (M)	0.050	0.0403 ± 0.0002	0.0403 ± 0.0003	1.380	1.381	12.706	12.706
	0.102	0.0413 ± 0.0005	0.0414 ± 0.0004				

[c]Mean value of [HCl] ± SD from five replication, [HCl]: concentration of HCl in molarity (M)

Table 5: The uncertainty of the proposed indicator

Parameter	SD of [HCl]		Uncertainty of [HCl]	
	PSPP-MG	PSPP-AG	PSPP-MG	PSPP-AG
Ur	0.0004	0.0004	0.000905	0.000905
UR	0.0001	0.0002	0.000226	0.000452

[HCl]: concentration of HCl in molarity (M)

Table 6: HCl content of sample

Proposed indicator	NaOH ± SD[d] (mL)	Linear regression equation	[HCl]
PSPP-MG	4.0214 ± 0.03	y = 98.285x + 0.0604	0.0403
PSPP-AG	4.0286 ± 0.03	y = 98.316x + 0.0603	0.0404

[d]Mean value of NaOH ± SD from seven replications, [HCl]: concentration of HCl in molarity (M)

Table 7: HCl content determined by titration with the proposed indicators and the standard indicators

Indicator		NaOH ± SD[d] (mL)	[HCl] ± SD[e]
Proposed indicator	PSPP-MG	4.0214 ± 0.0267	0.0410 ± 0.0003
	PSPP-AG	4.0286 ± 0.0267	0.0411 ± 0.0003
Standard indicator	Phenolphthalein	4.0214 ± 0.0393	0.0410 ± 0.0004
	Methyl red	4.0143 ± 0.0378	0.0409 ± 0.0004

[d]Mean value of NaOH ± SD from seven replications, [e]Mean value of [HCl) ± SD from seven replications, [HCl]: concentration of HCl in molarity (M)

HCl content determined using the proposed indicators and standard indicators

In this study, the HCl content in the sample was determined using the proposed indicators (PSPP-AG and PSPP-MG) and standard laboratory indicators (phenolphthalein and methyl red). The HCl content was calculated and shown in Table 7. It was observed that HCl content determined by the four indicators had the same concentration of 0.041 M. The HCl content calculated using the linear regression equation (Table 6) gave the same value as that calculated using the equation for determining the analyte concentration based on the titration data in Table 7. The results of the study have shown that the proposed indicators can serve as alternative to the standard laboratory indicators for HCl quantification in test samples.

Conclusion

PSPP as a TI for the quantification of hydrochloric acid in pharmaceutical dosage forms was successfully validated. The proposed indicators (PSPP-AG and PSPP-MG) have high linearity, with R^2 values of 0.9990 and 0.9991 for PSPP-AG and PSPP-MG, respectively. PSPP-AG and PSPP-MG have high accuracy, with a recovery of 102.4% for both. They also have high precision, with CV value ranging from 0.1609% to 0.8773%. The LoD of PSPP-AG and PSPP-MG were 0.0220 M and 0.0213 M, respectively, and the LoQ were 0.0734 M and 0.0709 M for PSPP-AG and PSPP-MG, respectively. The proposed indicators have good reproducibility (with CV ranging from 0.199% to 0.518%) and low uncertainty (ranging from 0.000262 to 0.000905 M). PSPP-AG and PSPP-MG are robust indicators. Variations in treatment did not affect their performance as indicators, where the t-stat < t-table of 3.475 < 4.303 was obtained for variations in sample volume and 1.380 < 12.706 for variations in NaOH concentration. The findings from this study also show that the hydrochloric acid content quantified with the proposed indicator was the same as that obtained with the standard indicators. Thus, the proposed indicators have good performance and can be used for the quantification of hydrochloric acid in pharmaceutical dosage forms. The method can also be adopted or adapted for practical experimental purposes. PSPP-AG and PSPP-MG can also be studied further to quantify hydrochloric acid in other samples.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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