
VALIDATION OF SUGAR DETERMINATION IN THE FCV TOBACCO LEAF USING FENOL-SULFAT METHOD BY SPEKTROFOTOMETER UV-VISIBLE

Siti Saroh, Maritha Nilam Kusuma

¹UPT. PSMB-Surabaya Tobacco Institute, Surabaya., Indonesia

²Adhi Tama Institute of Technology Surabaya, Indonesia

Email: saroh.tanwir@gmail.com, Maritha.kusuma@itats.ac.id

ABSTRACT

KEYWORDS

Validation method, total sugar, tobacco, phenol sulfate.

Virginia Flue Cured Tobacco is one of the type a lot of tobacco cultivated in Indonesia, Tobacco this many used as ingredient raw cigarette cigarettes, pipe tobacco and tobacco Susur (chewing tobacco). Leaf tobacco contain many compound very decisive chemistry the quality and taste of the resulting cigarettes, one of which is is sugar. Sugar content in tobacco give mild taste in cigarettes, but if rate too tall precisely will causes burning and irritation esophagus. However Thus, in SNI-01-4401-2006 it is not yet formulated method testing total sugar content. On research this conducted validation determination total sugar content in FCV tobacco with method phenol-sulfate by UV-Vis spectrophotometry aimed at get valid method for determination total sugar content of Virginia Flue Cured tobacco. Validity method showed through the parameters of linearity, precision, determination of LOD and LOQ, and accuracy. Test started with hydrolysis tobacco use sour 4.5% sulfate. Solution extract be read with UV-vis spectrophotometer at a wavelength of 490 nm with dye phenol-sulphate. Linearity test results curve obtained value of $r = 0.9997$ in the range work 20 – 100 mg/L, accuracy test results with 7 repetitions obtained the value of % recovery is 98.19 – 101.62%, LOD and LOQ test results respectively of 1,009 mg/L and 3,362 mg/L, while precision test results obtained deviation relative standard of 1.025%, result this still under the value of $2/3$ CVH is 1.86. From the results of the t-test obtained score tcount of 0.85, the result this moresmall from table ie 2.18. Validation result method for all parameters tested has Fulfill condition acceptance so that method phenol sulfate could used in determining rate glucose FCV tobacco routine

INTRODUCTION

Tobacco is commodity agriculture that has role strategic and value economical high in Indonesia. Tobacco plays a big role in determine development of the cigarette industry. (Murdiyati & Basuki, 2011). Industry tobacco also provides significant contribution for Indonesian economy, especially excise and foreign exchange, the field work, source income and development area (Handayani, Amrullah, Tarnanda, & Rahayu, 2018).

one type a lot of tobacco cultivated in Indonesia is tobacco virginia from from the United States. Plant it's so easy found in Lombok, West Nusa Tenggara and Jember East Java. Tobacco virginia is also known with the name Virginia Flue Cured (FCV), because of the drying process use Genre air heat in the oven (curing barn) (Peedin, 1999).

In the international world, tobacco virginia many traded in form Corrosion FCV or more known as virginia FC. Where are some big used as ingredient raw cigarettes (cigarettes) and some small for pipe tobacco and tobacco Susur (chewing tobacco).

Meanwhile, in Indonesia, tobacco Virginia FC a lot used in form cigarette blending white and kretek cigarettes, for shag (Murdiyati & Basuki, 2011).

Between compounds that affect quality product tobacco is sugar content in leaf tobacco, because that in SNI-01-4401-2006 regarding Virginia Flue Cured Tobacco, sugar content included in recommended compound for tested. Sugar content in tobacco affect the taste of the resulting cigarette (Herwati & Rochman, n.d.). Leaf sugar content tobacco give mild taste in cigarettes, but if rate too tall precisely will causes burning and irritation esophagus. (Dendo et al., 2018).

However Thus, in SNI-01-4401-2006 it is not yet formulated method testing total sugar content. Method test used During this use Luff Schrool method according to SNI-01-3996-1995 regarding DFC tobacco that counts reducing sugar content from type monosaccharides, although Tobacco also contains compound carbohydrate complex in form starch, cellulose, pectin, and sugar in high numbers (Tirtosastro & Murdiyati, 2010). So that for break compounds the Becomes monosaccharide needed long time and material chemical in amount big, so rated not enough economical.

Because of that required development method testing rate total glucose which has accuracy and precision high, detection limit low and cost operational inexpensive so that testing more efficient (Nur & Apriana, 2013). On research this conducted validation determination rate total glucose in leaves Virginia Flue Cured tobacco with method phenol the usual sulfate (Dubois) used in the determination of total sugar in the extract plants and food. Determination sugar level done based on measurement absorbance solution that has been reacted with phenol and sulfate using a UV-Visible spectrophotometer instrument at a length of the 490 nm wave (Djajadi & Hidayati, 2017).

Because Dubois' method is n't method raw for testing tobacco so laboratory must To do validation method for ensure suitability show work method that. This thing in accordance with clause 5.4.5.2 ISO/IEC 17025: 2017 which states that "Laboratory" must validate method no standard, designed / developed method laboratory, method standard used outside room intended scope, and affirmation as well as modification from method raw for confirm that method that in accordance for intended use"(Tirtosastro & Musholaeni, 2017). Because of that validation method analysis is very important done. With use method that has been validated so validity results measurement could accountable so that could used as base in calculation next (Sugihartini, Fudholi, Pramono, & Sismindari, 2012).

Validation method defined as evaluation to something method through test laboratory for prove that something method testing could give valid result with look at certain parameters (Hutagaol & Niken, 2017)

Parameters validated in research this is linearity, LOD, LOQ, and accuracy . Test results with method phenol-sulfate then compared with results sugar content with SNI-01-3996-1995 method using student's t. test (Indriana, 2016).

METHOD RESEARCH

Research Time and Location

Study this held from week second September 2022 to with week first month October 2022 at the Laboratory of the Testing Technical Implementation Unit Certification Quality Goods - Surabaya Tobacco Institute (UPT. PSMB-LT Surabaya) which is located on Jalan Gayung Bonsari In Number 12 A Surabaya.

Tools and Materials

Equipment used in research this is a shimadzu brand UV-Vis spectrophotometer instrument, a set of tools reflux equipped refrigeration, equipment glass, 25 mesh sieve, scales metler brand electronics toledo, a set of tools distillation, measuring pipette, and flask measure. Materials used in determination rate glucose this is ingredient standard glucose (Merck), aquadest, acid sulfate concentrated (Merck), and phenol (Merck).

Preparation Sample

Sample Virginia Flue Cured tobacco in form dry laminate cut small and blended until Becomes flour, then sifted with sieve size 25 mesh.

Preliminary Test

leaf sugar content test was carried out FCV tobacco with the SNI-01-3996-1995 method was repeated 7 times. The test result data is used as comparison test results using method phenol-sulfate through the t-test, that is compare second mean value method is different by significant or no with see value of t count and t table.

Hydrolysis Leaf Tobacco

Hydrolysis sample tobacco conducted use solution sour sulfate 4.5% (Nugroho nd) A total of 3 grams sample entered in pumpkin reflux and added 100 ml of acid 4.5% sulfate, then heated at 100oC for 1 hour. Next solution extract filtered and diluted in a 100 ml flask. Pipette 1 mL solution extract dilute in 100 mL flask using aquadest. Do replication testing 7 times.

Making Solution Standard

Making solution parent glucose 1000 mg/L

Weighed 1000 mg standard glucose and dissolved with aquadest in pumpkin measure 1 liter.

Making row standard

Made row standard with concentration end of 20; 40; 60; 80; 100 mg/l with pipette solution parent 1000 mg/l 2 each; 4; 6; 8; 10 ml to in pumpkin measure 100 ml. Then diluted with aquadest until limit. Made blank with treatment same without addition solution standard

Validation Parameters

Linearity

Pipette 1 ml of each solution row standard, add 1 ml of 5% phenol and shaken. After that Add 5 ml of acid sulfate concentrated with fast. Solution silence for 10 minutes, shaken and placed in hot water bath for 15 minutes, then be measured absorbance solution use UV - Visible spectrophotometer at long the 490 nm wave. Note absorbance of each series and make curve calibration with plotting score concentration (x) versus absorbance (y). From the data done calculation for get equality regression and coefficient correlation (r).

Detection limit and quantization limit

detection limit (LOD) and quantization limit (LOQ) tests were carried out for determine limit Lowest concentration standard that can be analyzed by qualitatively (LOD) and quantitative (LOQ) (Anonymous, 2019). LOD and LOQ values in research this calculated based on standard deviation response analyte and slope curve calibration (slope). LOD and LOQ explained in accordance equation (1) as following:

$$Q = (k \times S_b) / S_1, (2)$$

Where :

Q : LOD or LOQ

k : 3 for LOD and 10 for LOQ

Sb : deviation raw response analytic from blank

S1 : the direction of the linear curve Among response with concentration

S1 value is the same with score direction slope curve (slope) of equality regression $y = mx + n$, i.e. m value . Whereas deviation raw blank (Sb) value same with deviation residual standard (Sy/x), so that formula LOD and LOQ calculations become in accordance equations (2) and (3) as following:

$$\text{LOD} = (3 \times S(y/x))/\text{Slope}, (2)$$

$$\text{LOQ} = (10 \times S(y/x))/\text{Slope}, (3)$$

Precision test

Pipette 1 ml of the sample that has been hydrolyzed then 1 ml of 5% phenol was added and shaken. Add 5 ml of acid sulfate concentrated by quickly and let stand 10 minutes. Solution shaken and placed in a water bath filled with warm water for 15 minutes. Then measuring absorbance solution use UV -Visible spectrophotometer at a wavelength of 490 nm. Absorbance result note and do calculation rate glucose in tobacco use equality regression on determination linearity. Calculation result rate glucose with repeat 7 times, then used for calculation rpitability that is with calculate Horwitz CV where range acceptance RSD repeatability $< 2/3$ CVH.

Accuracy and precision

Test conducted with glucose spike method pure to in sample, that is with add as much 0.3 gram glucose or equivalent + 80% content glucose in sample. Then samples that have been dispiked analyzed with treatment same like sample on determination precision. Glucose levels in the spiked sample then compared with rate glucose sample pure and calculated acquisition return of added spikes in accordance equation (4).

$$\text{RECOVERY} = (A - B)/C \times 100\%, (4)$$

Where :

A : Concentration spiked sample

B : concentration sample

C : Concentration spike

Criteria gain % recovery received if results analysis give acquisition return in accordance table 1.

Concentration spike in the matrix sample, %	% Earnings return
100	98 - 102
> 10	98 - 102
> 1	97 - 103

> 0.1	95 – 105
0.01	90 – 107
0.001	90 – 107
0.0001	80 – 110
0.00001	80 – 110
0.00001	60 – 115
0.0000001	40 – 120

Source (Harmita, 2004: 119)

Description: at least conducted repetition as much as 5 times each example and done addition analyte as much as 50 - 150% levels analyte sample.

Significance test

Test significance two average of results testing sugar content with method phenol-sulphate and the method of SNI-01-3936-1995 was carried out by t test using formula in accordance equation (5) and (6) as following:

$$s^2 = \frac{(n_1 - 1) s_1^2 + (n_2 - 1) s_2^2}{(n_1 + n_2 - 2)} \quad (5)$$

$$t = \frac{(x_1 - x_2)}{(s \sqrt{1/n_1 + 1/n_2})} \quad (6)$$

Where :

S² = variance combined from two data

n₁, n₂ = number of data

x₁, x₂ = mean value

t = value tcount

degree freedom (v) = n₁ + n₂ - 2 (Miller 1991).

RESULTS AND DISCUSSION

Preliminary Test

preliminary test conducted determination rate glucose leaf tobacco with SNI-01-3996-1995 method regarding Tobacco Boyolali Bye. Test conducted as many as 7 repetitions and obtained the appropriate data table 2.

Table 2
Glucose Level Leaf FCV Tobacco Method
 SNI-01-3936-1995

Test	Glucose level (%)
1	11.03
2	11.24
3	11.11
4	10.95
5	11.01
6	10.84
7	10.98
Average	11.02
SB	0.13

% SBR	1,141
<i>Horwitz . CV</i>	2.79
<i>2/3 CV Horwitz</i>	1.86

From the data in table 2 it can be seen rate average glucose in leaves tested tobacco with the SNI method of 11.02. Precision testing with method this could received because %SBR value $< 2/3$ CVH. The average sugar content value obtained assumed as score reference because testing conducted with method standard for testing tobacco sugar content though no tobacco with varieties same.

Validation Parameters Method

Linearity curve standard

Determination linearity conducted with measure absorbance every solution in row standard. Absorbance value obtained used for make curve calibration, that is with plotting score absorbance solution standard (y) against concentration solution standard (x). From calculation this obtained equality regression and coefficient correlation (r). Absorbance data results measurement row standard glucose could seen in table 2.

Table 2
Absorbance data standard glucose

Concentration (mg/L)	Absorbance (y)
20	0.0810
40	0.1686
60	0.2525
80	0.3443
100	0.4201

The data in table 2 then made chart curve calibration shown in figure 1 .

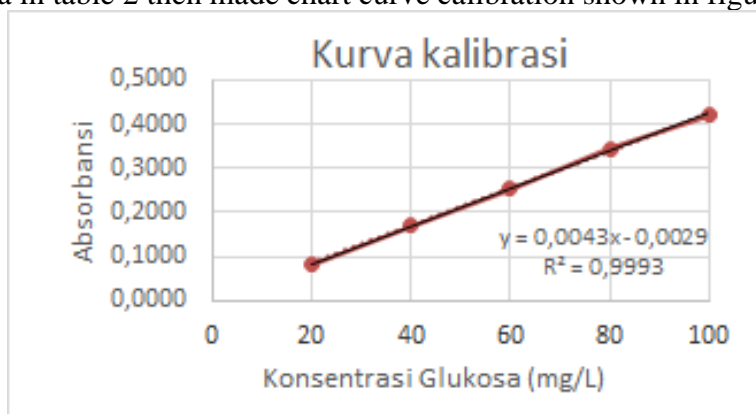


Figure 1
curve calibration solution standard glucose

Figure 1 shows equality regression linear $y = 0.0043x - 0.0029$, where slope (m) = 0.0043 and intercept (n) = -0.0029 with coefficient correlation (r) = 0.9997. Coefficient value correlation (r) obtained show score good because enter in range acceptance score coefficient correlation (r) is $r > 0.995$ so that linearity could accepted.

Calculation limit detection and limit quantization

Detection limit (*LOD*) is limit concentration analyte the smallest still could detected but no could set as score true, while limit quantization (*LOQ*) is limit concentration analyte the smallest you can quantized (Kan K 02, 2019).

LOD and *LOQ* values in research this calculated based on standard deviation response analyte and slope curve calibration (*slopes*). From calculation use equality linear $y = 0,0043x - 0,0029$ regression obtained score deviation residual standard (Sy/x) is 0.00144 so that obtained results *LOD* = 1.009 mg/L and the value of *LOQ* = 3.362 mg/L as shown in table 3.

Table 3
LOD and LOQ . calculation results

parameter	Results
r	0.9997
slope	0.0043
<i>LOD</i>	1.009
<i>LOQ</i>	3,362

Precision test

Precision test aim for see proximity individual test results on testing repeated with see scatter results measurement to the average yield measurement on sample homogeneous (Harmita 2004). Precision test carried out on research this is the repeatability test based on deviation raw relative (%) compared with score *CV Horwitz*.

Test conducted with 7 repetitions. Result data testing precision could seen in table

Table 4
Precision test results

Test	Absorbance	Glucose level (%)
1	0.1461	11.09
2	0.1420	11.31
3	0.1400	11.11
4	0.1392	11.06
5	0.1396	11.01
6	0.1386	11.04
7	0.1375	10.95
Average		11.08
SB		0.11
% SBR		1.025
<i>Horwitz . CV</i>		2.79
<i>2/3 CV Horwitz</i>		1.86

Based on the data in table 4, it is obtained %SBR value of 1.025 and still under the value of 2/3 CV Horwitz is 1.86 so that precision could accepted. There is relative test results on 7 repetitions more small from other test results possibility because error random including fluctuations temperature heating during the hydrolysis process and also when reading. Error random this could minimized with multiply repetition (Ariq, Afriani, Zuliandanu, & Suhartini, 2022)

Accuracy test

Accuracy the test result (accuracy) is requirements fundamental in testing. Test results with accuracy tall will give trust and validity results (Metode et al. 2020). Accuracy is level proximity Among score measurable with score actually. Accuracy could declared as percent acquisition return (*%recovery*) analyte added to the sample (Harmita 2004). Accuracy test this aim knowing degrees accuracy laboratory test results to score actually from sample (Ariq et al., 2022).

On research this is an accuracy test conducted with standard spike method to in sample tobacco *Virginia Flue Cured* that has been is known rate the glucose through precision test, then analyzed back. Accuracy test conducted 7 repetitions with concentration spiked standard about 90% rate glucose sample. Added analytes in the form of standard glucose pure purpose knowing influence treatment acid at level glucose standard. Calculation result accuracy shown in table 3.

Table 5
Level recovery test results glucose

Test	Sample rate	Spike Rate	Sample rate + spikes	% recovery
1	11.08	10.07	20.97	98.19
2	11.08	10.22	21.17	98.67
3	11.08	10,17	21.30	100.46
4	11.08	10.32	21.34	99.46
5	11.08	10.25	21.43	101.03
6	11.08	10,13	21.38	101.62
7	11.08	10,30	21.40	100.22
Range Recovery (%)			98.19 – 101.62	

From result the calculations contained in table 2 are obtained the % returns are in the range (98.12 – 101.62). This result Fulfill condition in accordance limit Acceptance of % recovery referred to by the laboratory of (98 – 102) % at spike concentration >10%. Difference in results acquisition return could caused existence error systematic origin from equipment, materials chemical, operational testing (Ariq et al., 2022)

Error systematic no could removed by overall, but could minimized, for example error from equipment could minimized with To do calibration tools used and errors personnel could minimized with enhancement competence personnel through training (Gandjar & Rohman, 2007).

Significance Test

Instrument statistics used in calculations validation method this is the student's t test for determine what is the average result of the sugar content test with the SNI-01-3936-1995 method and the average results of the sugar content test with method phenol-sulfate different by real. Hypothesis initial $H_0: \mu_1 = \mu_2$ or no there is difference results Among second method. The results of the t test calculation of both data from above test method could seen in table 6.

Table 6
Student's t test results

	SNI method	Method phenol-sulfate
\bar{x}	11.02	11.08

s	0.13	0.13
n	7	7
S_{total}	0.1198	
t_{hitung}	0.85	
$t_{(0,05;12)}$	2.18	

From result calculation obtained score $t_{count} < t_{table}$, so that could concluded second method give results rate glucose that is not different by significant so that could used in testing routine

CONCLUSION

Based on research that has been done, can concluded that testing sugar content in leaves FCV tobacco can conducted with method phenol-sulfate use UV-Visible spectrophotometer. Method has Fulfill requirements validation test method for linearity, precision, accuracy, limit parameters detection and limit quantization so that validity results the test could accountable.

REFERENCES

- Ariq, Muhammad Rizki, Afriani, Kartini, Zuliandanu, Denar, & Suhartini, Suhartini. (2022). Verifikasi Metode Uji Penetapan Kadar Tembaga (Cu) Dalam Air Permukaan Secara Spektrofotometri Serapan Atom. *Warta Akab*, 46(1).
- Dendo, Kasumi, Yugawa, Takashi, Nakahara, Tomomi, Ohno, Shin Ichi, Goshima, Naoki, Arakawa, Hirofumi, & Kiyono, Tohru. (2018). Induction Of Non-Apoptotic Programmed Cell Death By Oncogenic Ras In Human Epithelial Cells And Its Suppression By Myc Overexpression. *Carcinogenesis*, 39(2), 202–213.
- Djajadi, Djajadi, & Hidayati, Sulis Nur. (2017). *Pengaruh Pupuk Majemuk Terhadap Pertumbuhan, Produksi Dan Mutu Tembakau Cerutu Besuki No/Effect Of Compound Fertilizer On Growth, Yield And Quality Of Besuki No Cigar Tobacco*.
- Gandjar, I. G., & Rohman, A. (2007). *Analytical Pharmaceutical Chemistry*. Yogyakarta: Student Library, 222.
- Handayani, Sri Seno, Amrullah, Amrullah, Tarnanda, Riki, & Rahayu, Baiq Anita. (2018). Proses Degradasi Lignin Pada Limbah Batang Tembakau Sebagai Persiapan Produksi Bioetanol. *Jurnal Pijar Mipa*, 13(2), 140–146.
- Herwati, Anik, & Rochman, Fatkhur. (N.D.). *Kesesuaian Varietas Introduksi Tembakau Virginia Fc*.
- Hutagaol, Ricson P., & Niken, Niken. (2017). Validasi Metode Penetapan Kadar Cyanocobalamin Secara Spektrofotometri Visibel Double Beam. *Jurnal Sains Natural*, 2(1), 24–34.
- Indriana, Kovertina Rakhmi. (2016). Produksi Bersih Pada Efisiensi Dosis Pupuk N Dan Umur Panen Daun Tembakau Terhadap Kadar Nikotin Dan Gula Pada Tembakau Virginia. *Jurnal Agrotek Indonesia (Indonesian Journal Of Agrotech)*, 1(2).
- Murdiyati, A. S., & Basuki, Teger. (2011). *Agribisnis Tembakau Virginia. Monograf Balittas: Tembakau Virginia. Balai Penelitian Tanaman Tembakau Dan Serat*.
- Nur, Yudha Hadian, & Apriana, Devi. (2013). Daya Saing Tembakau Virginia Lokal Di Pasar Dalam Negeri. *Buletin Ilmiah Litbang Perdagangan*, 7(1), 73–90.
- Peedin, Gerald F. (1999). *Flue-Cured Tobacco Information*. North Carolina Cooperative Extension Service.

- Sugihartini, Nining, Fudholi, Achmad, Pramono, Suwidjiyo, & Sismindari, Sismindari. (2012). Validasi Metode Analisa Penetapan Kadar Epigalokatekin Galat Dengan Klt Densitometri. *Jurnal Ilmiah Kefarmasian*, 2(1), 81–87.
- Tirtosastro, Samsuri, & Murdiyati, A. S. (2010). *Kandungan Kimia Tembakau Dan Rokok*.
- Tirtosastro, Samsuri, & Musholaeni, Wahyu. (2017). Penanganan Panen Dan Pasca Panen Tembakau Di Kabupaten Bojonegoro. *Buana Sains*, 15(2), 155–164.

Copyright holders:

Ervina Anas Meliana, Naufal Febri Nugroho, Christina Dwi Astuti (2022)

First publication right:

Devotion - Journal of Research and Community Service



This article is licensed under a [Creative Commons Attribution- ShareAlike 4.0 International](https://creativecommons.org/licenses/by-sa/4.0/)