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Validation of Spectrophotometric Method for Analysis of Anionic Surfactant Dodecyl Benzene Sulphonate (DBS) in Catfish (*Clarias batrachus*) Using Malachite Green

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ABSTRACTS

Validation method of DBS anionic surfactant analysis on *Clarias batrachus* has been conducted. The method of analysis was divided into two-phase, namely the extraction with solid-liquid extraction using Soxhlet and analysis DBS. The extraction was performed using *n*-hexane and methanol for 9 and 6 hours, respectively. The analysis was performed using Spectrophotometer UV-Vis based on the complex formation of surfactant-malachite green (DBS-MG). These methods are applied to determine DBS accumulation of *Clarias batrachus* with DBS concentration exposure and DBS concentration of*Clarias batrachus* in markets.The result showed that the parameters of validation methods has high acceptability as linearity (R^2 = 0.99), limit of detection (LOD) and limit of quantification (LOQ) (0.029 mg/L and 0.089 mg/L), sensitivity ($\varepsilon = 38.15 \times 10^4$ L mol^{-1} cm^{-1}), precision (RSD = 0.10-1.83 %) and accuracy (recovery = 80-92 %). The result of analysis of DBS in *Clarias batrachus* with 2.5; 5; 10; 15 mg/L of DBS concentration exposure obtained 5.5; 6.8; 7.9; 8.7 mg/L respectively and *Clarias batrachus* from markets in a range 2.0-4.2 mg/L. The result showed that the analysis of DBS anionic surfactants using MG can be applied for *Clarias batrachus*.

Keywords: validation method, extraction, clarias batrachus, dodecyl benzene sulfonate, malachite green

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1. Introduction

Detergent products have been used by almost all the inhabitants for various purposes such as washing clothes and furniture as well as other cleaning materials. It is composed of surfactants, builder, and additive materials. Surfactants are most important than other material. In 2003, more than 18.2 million tons surfactants were produced and anionic surfactants are highest component of this production (Haunted, 2004). Anionic surfactants such as dodecyl benzene sulfonate (DBS) is the best product for cleaning so that most people using DBS as cleaner generally. Surfactants waste were throw out to the river directly by consumers. It caused contaminations of the river. A concentration of anionic surfactant in water river gave negative effect for aquatic organisms such as fish, mollusks, and other species. It has high possibility of accumulation by biota. The accumulation depends on bioconcentration factor (BCF) DBS value on biota. The BCF value of DBS in some aquatic organisms have been reported such as *macrobrachium rosenbergii* (Santoso, 2010), *lumbriculus variegatus* (Maenpaa and Kukkonen, 2006), *clarias batrachus* (Versteeg and Rawlings, 2003). Because of that, the population of aquatic organism has been a risk to accumulate of DBS. The number of DBS in the whole body of an organism depends on time exposure, DBS concentrate, and aquatic organisms condition. The accumulation of DBS in *Clarias batrachus* made decreasing of fish quality. It is caused by protein degradation ability of DBS is 1000 times more efficient than traditional denaturants such as urea and guanidium chloride (Otzen, 2011). The decreasing of fish quality would give direct negative effect to consumer.

Analysis of anionic surfactants in fish has been conducted. There is two major step for analysis DBS in fish. The first, extraction of anionic surfactants in fish. It is using usually Soxhlet, PLE (pressurized liquid extraction) (Martin *et al.*, 2006), automated soxhlet extraction, accelerated solvent extraction, ultrasound-assisted extraction, and supercritical fluid extraction. Soxhlet instrument is better to extract DBS from fish. That is because of simple procedure and cheaper (Munoz *et al.*, 2004; Jadhav *et al.*, 2009; Saez *et al.*, 2000).

The second, analysis of DBS from fish. There is some instrument has been recommended such as HPLC (high-performance liquid chromatography), TLC (thin layer chromatography), IE (ion chromatography) UV-Vis Spectrophotometry, mass Spectrophotometry, and IR Spectrophotometry (Zoller, 2005; Olkowska *et al.*, 2012; Kargosha *et al.*, 2007). The Using of UV-Vis Spectrophotometry is better than others, a cause of simple procedure and not expensive.

2. Materials and Methods

2.1. Chemical

Malachite Green (MG) oxalate, Sodium acetate, potassium dihydrogen phosphate, a buffer of acetate (pH 3, 4,

5, 6), chloroform, n-hexane, methanol, Sodium Dodecyl Benzene Sulfonate (DBS), and aqua bikes. All of the chemicals from Merck except DBS from Wako Pure Chem. Industries Ltd and aqua bikes from the chemistry laboratory.

2.2. Instruments

Soxhlet, UV Vis Spectrophotometer UV 1700 Shimadzu (E) 230 VCE, freeze dryer, pH meter, freezer, funnel 125 mL.

2.3. Sample Preparation

Samples of *Classics batrachus* from markets and breeding ponds was homogenized by a blender. Then it is pondered and put into the freezer before dehydration by freeze dryer. It is supposed to remove the interference of water while extraction process.

2.4. Optimation of Methods

2.4.1. Wavelength optimation

The Standard solution of DBS 0.1; 0.6; 0.9 was taken 20 mL respectively and put into funnel 125 mL, then 5 mL of MG 10 mg/L was added and the result of a reaction was extracted by chloroform 5 mL. Organic phase from extraction result was analyzed by UV-Vis Spectrophotometer at 500-750 nm.

2.4.2. Optimation of pH

The standard solution of MG 10 mg/L with different pH was made by using acetate buffer. It has four pH variation, pH 3; 4; 5; and 6. Standard solution of MG 10 m/L pH 3; 4; 5; and 6 was taken 5 mL respectively and reacted with 20 mL standard solution of DBS 0.6 mg/L. Then the result of a reaction was extracted by chloroform 5 mL. Organic phase from solution was analyzed by UV-Vis spectrophotometer at 623 nm.

2.4.3. Concentration DBS: MG optimization

The standard solution of MG 1; 5; 10; 15; 20; 30 mg/L pH 5 was taken 5 mL respectively and reacted with 20 mL standard solution of DBS 0.6 m/L. Then it was extracted with chloroform 5 mL and analyzed by UV-Vis spectrophotometer at 623 nm.

2.4.4. Time stabilization complex of DBS-MG

The standard solution of MG 10 m/L pH 5 took 5 mL and reacted with 20 mL DBS 0.6 mg/L. Then it was extracted with chloroform 5 mL. Organic phase from a result of extraction was analyzed by UV-Vis spectrophotometer at 623 nm with different times.

2.5. Analysis of analytical parameters

2.5.1. Linearity

The standard solution of DBS 0.1; 0.2; 0,4; 0.6; 0.8; and 0.9 mg/L was taken 20 mL respectively and reacted with 5 mL MG 15 mg/L. Then it was extracted with chloroform 5 mL. The absorbance of the organic phase was measured by UV-Vis spectrophotometry at 623 nm.

2.5.2. Limit of detection (LOD) and limit of quantification (LOQ)

The determination of LOD and LOQ have been conducted and it was determined by calculation. It has the same procedure with linearity procedure.

2.5.3. Sensitivity

The determination of sensitivity has been conducted based on savin formulation. Savin (1979) said that sensitivity value equal with a slope of the calibration curve of concentration (moles/L) and absorbances. The data from the calibration curve has been converted to sensitivity.

2.5.4. Precision

The analysis of precision (intraday precision nor interday precision) has been determined. The standard solution of DBS 0.1; 0.6; and 0.9 was taken 20 mL respectively and reacted with 5 mL MG 15 mg/L. Then it was extracted with chloroform 5 mL. Absorbances of the organic phase were measured with a different time in the same day (intraday precision) and different day (interday precision) by UV-Vis spectrophotometer at 623 nm.

2.5.5. Accurate

The determination of accurate was conducted by spiking methods. Standard solution of DBS 0.1; 0.2; 0.4; 0.8; and 0.9 mg/L was taken 15 mL respectively and it was added to fish sample (exposure 0 mg/L DBS). A solution of samples after extraction procedure by soxhlet was reacted with 5 mL MG 15 mg/L pH 5. Then it was extracted by chloroform 5 mL. Absorbances of the organic phase were measured by UV-Vis spectrophotometer at 623 nm.

2.6. Analysis of sample

The fish samples from the breeding ponds at Fishering Faculty facility, University of Gadjah Mada (UGM) with DBS exposure 0; 2,5; 5; 10; and 15 mg/L for 3 months and fish samples from markets were homogenized and put into the freezer before freeze dryer process. Then samples from freeze dryer process were taken and extracted by Soxhlet. The soxhlet process using n-hexane for 9 hours and methanol for 6 hours. Analyte from methanol solvent was then evaporated and redissolved by warm aquabides to made 100 mL samples solution. Then samples solution was reacted by MG and extracted by chloroform. This procedure followed the result of optimation methods.

3. Results and Discussion

3.1. Optimation of Methods

3.1.1. Optimum of wavelength

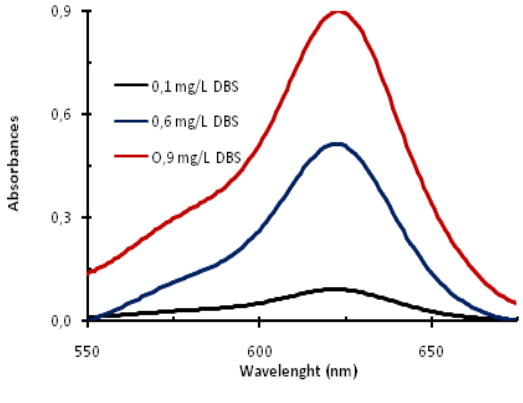

Fig. 1. The determination of λ max

Based on data reported on Fig. 1, the maximum absorbances of samples occur at 623 nm. The analysis of anionic surfactant using MG has maximum absorbance at 621 nm. This result shows the transition of an electron from ground state $\pi \rightarrow \pi^*$ in complex DBS-MG compound (Kargosha et al., 2007).

3.1.2. Optimation of pH

Data reported in Fig. 2 is an optimation of MG pH solution. The maximum absorbances occurred at wavelength of 623 nm of MG solution pH 5 with absorbances equaled to 0.554.

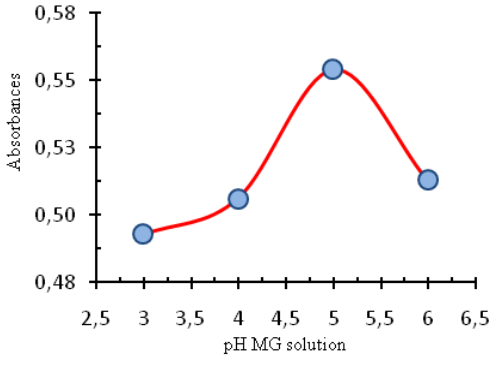

Fig. 2. Optimation of pH MG solution

The optimation results of the MG solution acidity showed the highest ionization of MG solution occurred at pH 5. As shown on Fig. 2, the absorbances of the MG solution before and after pH 5 are lower than the absorbance occurring at the pH 5. The absorbance decrease means low ionization of the MG solution occurred at pH before and after 5 (Huang and Zhang, 2006). This data conclusively indicated that DBS-MG complex markedly formed at pH 5.

3.1.3. Optimation of DBS: MG concentration

Based on Table 1, maximum absorbance was showen by comparison of moles DBS: MG is 1:2 with absorbance 0.688 at 623 nm. It means DBS is extracted completely by MG.

Table1. Optimation of moles comparison DBS: MG

Concentration of DBS (mg/L)	Mole of DBS (µmol)	Concentration of MG (mg/L)	Mole MG (µmol)	Mole comparation of DBS:MG	Absorbances
0.6	0.0344	1	0.0045	1:0.1	0.054
0.6	0.0344	5	0.0224	1:0.6	0.491
0.6	0.0344	10	0.0447	1:1.3	0.556
0.6	0.0344	15	0.0671	1:2	0.688
0.6	0.0344	20	0.0894	1:2.6	0.687
0.6	0.0344	30	0.1342	1:4	0.688

3.1.4. Time stabilization complex of DBS-MG

As shown on Fig.3, the DBS: MG complex has already been stabil at the contact time of 20 min. The range unstability time of DBS-MG complex between 120-140 min indicated the photodegradation or photolysis effect (Perez *et al.*, 2008). The photodegradation effect cause the DBS-MG complex form a new compund and change the maximum absorbance.

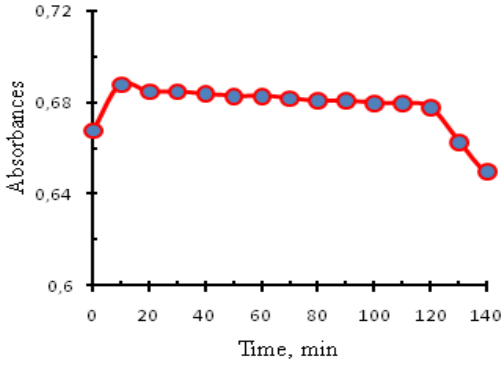

Fig.3. Optimation of the stable contact time of DBS: MG complex

3.2. Analyses of analytical parameters

3.2.1. Linearity

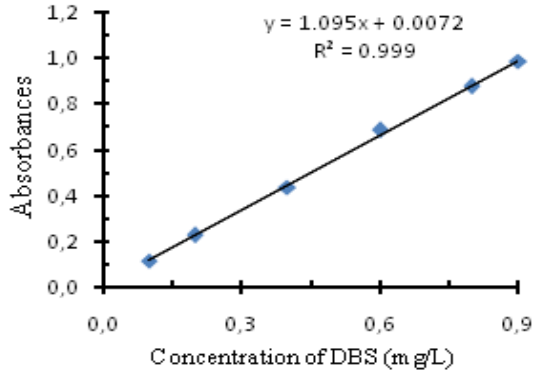

Fig. 4. Linearity curve

Base on the Fig. 4, the linearity parameter indicate that R^2 = 0.999 and linear equation y = 1.095x + 0.0072. The R^2 value reported indicates a good linearity (Schneider et al., 2010).

3.2.2. Limit of detection (LOD), limit of quantification (LOQ), and sensitivity

Base on data of the calibration curve, the value of LOD, LOQ, and sensitivity ε is 0.0293 mg/L, 0.089 mg/Land 38.15 × 10^4 L mol^{-1} cm^{-1}, respectively. These data indicate that analysis of ionic DBS surfactants by spectrometry method has a good performance which involved the LOD, LOQ, and sensitivity which is in agreement with data reported by another author (Savin, 1979).

3.2.3. Precision

Based on provisions of Horwitz function and AOAC which stated that analyte concentration below 1 mg/L has precision values (%RSD) received: <16% and <11 %, respectively. The resulted data from the analysis of the precision involving intraday precision reported in Table 2 and inter-day precision reported in Table 3.

3.2.4. Accuracy

The Accuracy of the method is shown on Table 4. Accuracy is expressed as % recovery and determined by spiking method. Base on the data, the accuracy parameter was acceptable because it corresponds to analyte concentration level as proposed by Gonzalez *et al* (2010).

Table 2. The intraday preccision data

Concentration of DBS (mg/L)	Absorbance, λ_{max} = 623 nm				% RSD
	Day I	Day II	Day III	Average	
0.1	0.118	0.114	0.117	0.116	1.83
0.6	0.687	0.684	0.688	0.686	0.31
0.9	0.986	0.985	0.988	0.986	0.16

Table 3.The interday precision data

Concentration of DBS (mg/L)	Absorbances, λ_{max} = 623 nm				% RSD
	I	II	III	Average	
0.1	0.117	0.115	0.115	0.116	1.05
0.6	0.686	0.684	0.686	0.685	0.18
0.9	0.988	0.987	0.986	0.987	0.10

Table. 4. Accuracy of analytical method

DBS Spiking (mg/L)	Absorbances, (λ = 623 nm)				% recovery
	I	II	III	Average	
0.015	0.018	0.015	0.016	0.016	80
0.030	0.030	0.028	0.028	0.029	87
0.060	0.053	0.051	0.052	0.052	89
0.120	0.096	0.095	0.096	0.096	88
0.150	0.108	0.109	0.109	0.123	92

Table 5. Analysis of DBS on the fish sample

DBS (mg/L)	Wet mass (g)	Absorbances	Concentration	
			mg/L	mg/g
P 2.5	182.062	0.590	5.3	0.003
	95.230	0.615	5.5	0.006
	180.130	0.632	5.7	0.003
P 5	187.643	0.741	6.7	0.003
	157.314	0.762	6.8	0.004
	203.776	0.773	6.9	0.003
P 10	97.381	0.878	7.8	0.008
	88.702	0.870	7.8	0.009
	64.578	0.884	8.0	0.012
P 15	88.875	0.952	8.6	0.010
	49.117	0.964	8.7	0.018
	82.365	0.981	8.9	0.011

Samples from markets at Yogyakarta

Table 6. Analysis of DBS on samples from markets at Yogyakarta

Markets code	Absorbances, (λ= 623 nm)				Concentration	
	I	II	III	Average	mg/L	mg/g
PSRB	0.474	0.473	0.476	0.474	4.2	0.007
PSRC	0.225	0.224	0.225	0.225	2.0	0.002
PSRJ	0.283	0.285	0.285	0.284	2.5	0.004
PSRK	0.250	0.247	0.246	0.248	2.2	0.004
PSRP	0.346	0.347	0.347	0.347	3.1	0.005

3.3. Analysis of fish samples

Samples were taken from breeding ponds. DBS metabolism in catfish can form a new compound carboxylic sulfophenyl (SPC) with a negative charge (Leon et al., 2006). The analysis of DBS using MG shows that the metabolism process of DBS on living organism need more time to transformed all DBS to SPC. Base on data on Table 5 showed that the increase of concentration exposure 2.5-15 mg/L has a linearity relation with the concentration of DBS in the whole body of catfish.

Analysis DBS using MG is also used to the sample from a traditional market in Yogyakarta. The result showed in table 6 that samples of catfish has smaller concentration than the samples from breeding ponds even though it is indicated that

the catfish sold in a traditional market were bred in a polluted environment with DBS.

4. Conclusion

The analytical parameter of validation method have acceptability as linearity (R^2 = 0.999), limit of detection (LOD), limit of quantification (LOQ) and sensitivity is 0,0293 mg/L, 0,089 mg/Land 38.15 × 10^4 L mol^{-1}, respectively, precision (RSD) 0.16-1.86%, and accuracy 80-92%.The catfish in breeding ponds and the sample from traditional market has a positive accumulation of DBS. The result analysis of DBS with concentration exposure equaled to 2.5; 5; 10 ; 15 is 5.5; 6.8; 7.9; 8.7, mg/L respectively, and the sample from traditional market was 4.2; 2.0; 2.5; 2.2; 3.1 mg/L. The result showed that method analysis of anionic surfactant DBS using dMG could be applied for catfish sample.

References

Gonzalez-Roca E, Garcia-Albéniz X, Rodriguez-Mulero S, Gomis RR, Kornacker K, Auer H. Accurate Expression Profiling of Very Small Cell Populations. PLoS ONE 2010; 5(12): e14418. **DOI: https://doi.org/10.1371/journal.pone.0014418**.

Haunted HG. Types and Typical Ingredients of Detergents, Marcel Dekker, New York. 2004.

Huang XL and Zhan JZ. Surfactant-sensitized malachite green method for trace determination of orthophosphate in aqueous solution. Analytica Chimica Acta 2006; 580: 55–67. **DOI: 10.1016/j.aca.2006.07.046**

Jadhav D, Rekha, B N, Gogate PR, Rathod VK. Extraction of Vanillin from Vanilla Pods: a Comparison Study of Conventional Soxhlet and Ultrasound Assisted Extraction, J. Food Engineer; 2009; 93: 421-426. **DOI: https://doi.org/10.1016/j.jfoodeng.2009.02.007**

Kargosha K, Ahmadi SH, Mansourian M, and Azad J. Simultaneous Determination of One Nonionic and Two Anionic Surfactants Using Forier Transform Infrared Spectrometry and Multivariate Analysis, Talanta; 2007; 75: 589-593. **DOI: https://doi.org/10.1016/j.talanta.2007.11.065**

Leon V M, Lopez C, Martin P A L, Prats D, Varo P, and Mazo EG. Removal of linear Alkylbenzene Sulfonate and Their Degradation Intermediates at low Temperatures During Activated Sludge Treatment. Chemosphere; 2006; 64: 1157-1166. **DOI: http://doi.org/10.1016/j.chemosphere.2005.11.045**

Maenpaa K and Kukkonen JVK. Bioaccumulation and Toxicity of 4-Nonylphenol (4-NP) and 4-(2-Dodecyl)-Benzene Sulfonate (LAS) in LumbriculusVariegatus (Oligochaeta) and ChironomusRiparius (Insecta), Aquat. Toxico;. 2006; 77: 329-338. **DOI: https://doi.org/10.1016/j.aquatox.2006.01.002**

Martin P A L, Parra AG, Mazo E G. Simultaneous Extraction and Determination of Anionic Surfactants in Waters and Sediments, J. Chromatogr *A*; 2006; 1114: 205-210. **DOI: https://doi.org/10.1016/j.chroma.2006.03.014**

Munoz D A, Saez M, Martin P A L, Parra AG, Mazo EG. New Extraction Method for the Analysis of Linear Alkylbenzene Sulfonates in Marine Organisms Pressurized Liquid Extraction versus Soxhlet Extraction, J. Chromatogr. A;, 2004; 1052: 33-38. **DOI: https://doi.org/10.1016/j.chroma.2004.08.014**

Olkowska E, Polkowska Z, and Namiesnik J. Review: Analytical Procedures for the Determination of Surfactants In Environmental Samples, Talanta; 2012; 88, 1-13. **DOI: https://doi.org/10.1016/j.talanta.2011.10.034**

Otzen, D. Protein-Surfactant Interaction: A Tale of Many States, Biochim. Biophys.Acta; 2011; 1814: 562-591.

Pérez ELA, Agüera A, Hernando MD, Malato S, and Fernández-Alba AR. Photodegradation of malachite green under natural sunlight irradiation: Kinetic and toxicity of the transformation products. Chemosphere. 2008; 70, 11: 2068-2075. **DOI: https://doi.org/10.1016/j.chemosphere.2007.09.008**

Saez M, Leon V M, Parra A G, and Mazo EG. Extraction and Isolation of Linear Alkylbenzene Sulfonates and Their Intermediate Metabolites from Various Marine Organisms, J. Chromatogr., 2000; 889: 99-104. **DOI: https://doi.org/10.1016/S0021-9673(00)00204-1**

Santoso L. KajianToksiksitas Dan Bioakumulasi Surfaktan Detergen Linear Alkilbenzen Sulfonate (LAS) Pada Udang Galah Macr obrachiumrosenbergi, Tesis, IPB, Bogor; 2010.

Savin S B. Fundamental of Analytical Chemistry, CRC Crit. Rev. Chem; 1979; 8: 55.

Schneider A, Hommel G, and Blettner M. Linear Regression Analysis. Dtsch Arztebl Int. 2010; 107(44): 776–782. **DOI: 10.3238/arztebl.2010.0776.**

Versteeg D J and Rawling J M. Bioconcentration and Toxicity of Dodecylbenzene Sulfonate (C_{12} LAS) to Aquatic Organisms Exposed in Experimental Streams, Arch. Environ.Contam.To xicol; 2003; 44: 237-246. **DOI: https://doi.org/10.1007/s00244-002-2017-2**

Zoller U. Handbook of Detergent Part C: Analysis, Marcel Dekker, New York; 2005.

Conflict of interest: Non declare