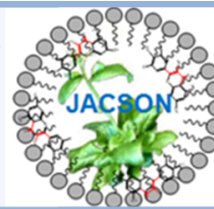


Content lists available at www.jacsonline.org/D176750082-LROR/ JACSONline GP, DOI:10.22341

Journal of Applied Chemical Science

Journal homepage: www.jacsonline.org/journals/jacson/



Validation of Analytical Method Determination of Sodium Dodecyl Benzene Sulfonate (DBS) in Catfish (*Clarias batrachus* L.) by Spectrophotometric Using Methylene Blue

*Christiani Dewi Q. M. Bulin¹, Adhitasari Suratman², and Roto²

¹Department of Chemistry, Faculty of Mathematical and Sciences, Widya Mandira Catholic University Kupang; ²Department of Chemistry, Faculty of Mathematical and Sciences, Gadjah Mada University – Yogyakarta, INDONESIA

Article history: Received in revised form 26-03-2018

Accepted 20-04-2018

Available online June 20, 2018

Cite this article as: Bulin CDQM, Suratman A, and Roto. *Validation of Analytical Method Determination of Sodium Dodecyl Benzene Sulfonate (DBS) in Catfish (*Clarias batrachus* L.) by Spectrophotometric Using Methylene Blue.* J Applied Chem. Sci. 2018, 5(1):414-417

DOI : <https://dx.doi.org/10.22341/jacs.on.00501p414>

p-ISSN: 2089-6328, e-ISSN: 2580-1953 © 2018 JACSONline GP. All right served



The JACSONline Group Publisher publishes the work of Jacson-Journal of Applied Chemical Science eISSN: 2580-1953/pISSN: 2089-6328 under the licensing of a [Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License](https://creativecommons.org/licenses/by-nc-sa/3.0/). Authors retain the copyright to their work. Users may read, copy, and distribute the work in any medium provided the authors and the journal are appropriately credited. The users may not use the material for [commercial purposes](#).

ABSTRACTS

A spectrophotometric method for analysis of DBS anionic surfactant in *Clarias batracus* has been validated. The method of analysis was divided into two phases. Extraction with solid-liquid extraction using Soxhlet and analysis of DBS. The extraction was performed using solvent of n-hexane and methanol for 9 and 6 hours, respectively. The analysis was performed using Spectrophotometer UV-Vis based on the complex formation of DBS-methylene blue (DBS-MB). This methods is applied to the determination of DBS in local catfish after DBS exposure and that of obtained in markets. The results showed that the parameters of validation methods have high acceptability as liniarity ($R^2 = 0.99$), limit of detection (LOD) and limit of quantification (LOQ) (2.93 mg/g and 9.75 mg/g), sensitivity ($\epsilon = 2.44 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$), precision (RSD = 0.14-1.38%) and accuracy (% recovery in a range 82-110 %). The results of analysis of DBS in catfish with 2.5; 5; 10; 15 mg/L of DBS concentration exposure are 0.87; 1.67; 8.50 dan 18.10 mg/kg, respectively and catfish from markets in a range 8.5-61 mg/kg. The result showed that the method of analysis of DBS anionic surfactant using MB could be applied for catfish samples..

Keywords: Validation metode, extraction, catfish, *Clarias batrachus*, dodecyl benzene sulfonate, methylene blue.

*Corresponding author: christiani.bulin@gmail.com

1. Introduction

A surfactant is a compound with hydrophilic and hydrophobic groups. The anionic surfactant sodium dodecyl benzene sulfonate (DBS) is a key raw material in the detergent and household cleaning agents (Schmitt, 2001). The analysis of anionic surfactant is generally conducted by UV-Vis spectrophotometry, GC-MS and HPLC (Traverso-Soto et al., 2012; Munoz et al., 2004).

The UV-Vis spectrophotometry is commonly used to perform the analysis of anionic surfactants in water samples. In this method, an anionic surfactant in the sample is reacted with a complexing cationic compound with the methylene blue as a complexing agent. DBS-MB complexes, extracted from the sample solution by using chloroform, were analyzed on a maximum wavelength (Chitikela et al., 1995; Jurado et al., 2006; Koga et al., 1999). This method require a validation in order to determine whether this method is able to analyze the anionic surfactant in living organisms.

The analysis requires the isolation of anionic surfactant from living organism prior to analysis. Isolation is often conducted by the method of Soxhlet and solid phase extraction (Saez et al., 2000), Soxhlet and pressurized liquid extraction (PLE) (Munoz et al., 2004), automated Soxhlet extraction, accelarated solvent extraction, ultrasound assisted extraction and supercritical fluid extraction (Olkowska et al.,

2012). The benefits of the Soxhlet extraction method are more efficient and more economical with methanol as a solvent.

In this study, the validation of analytical method determination of DBS in living organism was performed which is to evaluate the possibility use of the analytic method for determination of anionic surfactant DBS in aquatic organisms. The samples were obtained from a full setup catfish exposed by anionic surfactant DBS and catfish in the traditional market in Yogyakarta. An anionic surfactants exposure is known to cause accumulation in some aquatic organisms such as prawns (Santoso, 2010), fathead minnow fish (Tolls, 1997), *Hyalella azteca*, *Corbicula fluminea* clam and catfish (Versteeg and Rawlings, 2003). DBS accumulated in catfish was suspected to reduce their qualities as nourishment, caused by the capability of an anionic surfactant in degrading protein 1000 times faster than urea and guanidium chloride (Otzen, 2011).

2. Materials and Methods

2.1 Materials

The materials used in this study were Sodium DBS purchased from Sigma-Aldrich; methylene blue, phenol-phtalein, sodium dihydrogenphosphate monohidrat, sulphate acid, chloroform, ethanol, hexane, methanol, and sodium hidroxide were purchased from Merck. The equipments include: laboratory glass-wares, Soxhlet extraction set, pH meter, analytical balance, rotary evaporator, refrigerator,

freeze dryer, and UV-Vis spectrophotometer (UV-Vis 1700 type).

2.2 Experimental Procedures

2.2.1 Sample preparation

Catfish were raised in tanks. Five tanks were used, each for 30 catfish. Sodium DBS were added to each tank before putting the catfish and the concentrations were set to 0; 2.5; 5; 10 and 15 mg/L. After three months, the catfish were ready to be analyzed. The catfish that raised in tank without Sodium DBS were used as a sample for the validation, whereas the catfish with Sodium DBS used as samples to compare with the sample from traditional market in Yogyakarta.

The catfish were netted out from the tank, subsequently killed and stored at -20°C . After being chopped, the catfish was homogenized and frozen. The frozen samples were lyophilized using freeze dryer, weighed again and grounded in a mortar with a pestle.

2.2.2 Soxhlet extraction

Samples were extracted using the method proposed by Saez et al.(2000). The method was started by filling the washed extraction timbles with each sample (≈ 100 g). Then, sample were extracted in a shoxlet apparatus with 250 mL n-hexane for 9 hours followed by 250 mL methanol for 6 hours. Afterwards, the extract was evaporated in evaporator and the dried residue was redissolved with 100 mL water.

2.2.3 Spectrophotometric procedure and validation characteristic

The spectrophotometric method was carried out according to the standard method used in Indonesia. A total of 50 ml of sampel and 12.5 mL of methylene blue were put in a 100 mL separating funnel. The mixture was extracted with 5 mL chloroform. The mixture was shaken for 30 seconds, then allowed to stand until separation occurs. The chloroform phase was taken and placed into another separating funnel. The aqueous phase was re-extracted by adding 5 ml of chloroform and shaken for 30 seconds. Then all the chloroform phase were collected together. This step was repeated once more and the collected chloroform phase was extracted after adding a 25 mL washing solution. The mixture was shaken for 30 seconds and the chloroform phase was taken to be measured with the spectrophotometer at 652 nm.

Validation characteristics which have been evaluated were accuracy, precision, detection limit, quantification limit, sensitivity, linearity and range. The accuracy of this method was expressed as percent of recovery and determined by the spiked-placebo recovery method. The precision was expressed as repeatability covering an intraday precision and an interday precision. The range was depend on the linearity of concentration which have been tested i.e. 0.05; 0.2; 0.4; 0.8; 1.0; 1.2; and 1.6 mg/L. Limits of detection and quantification were determined based on the standard deviation (S) and the slope (m) of the calibration curve. A calibration curve was made using a sample containing the analyte DBS refers to a

standard calibration curve DBS. Sensitivity was determined by using the standard curve equation.

3. Results and Discussion

3.1 Validation characteristics

3.1.1 Linearity

The data showed that the curve is linear with a correlation coefficient of 0.9987 and linear equation, $y = 0.6992x + 0.1036$. Based on the correlation coefficient (R^2) obtained, the equation can be categorized as good linear regression equation ($R^2 \geq 0.997$) (Harsojo, 2012). This indicated DBS surfactant analysis can be conducted in the DBS concentration range of 0.025 to 1.6 mg/L.

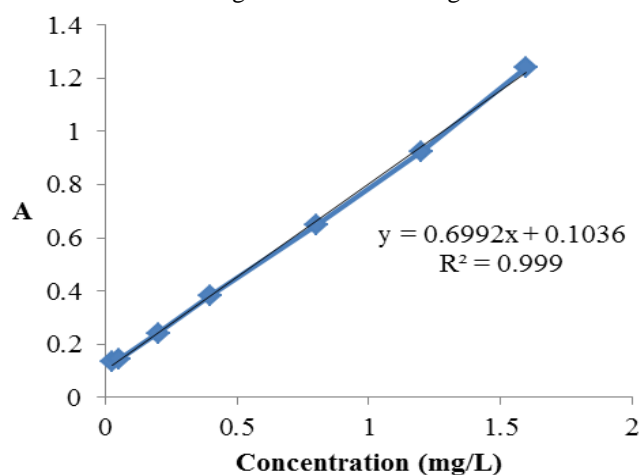


Fig. 1. Calibration curve of DBS-MB

3.1.2 Limit of detection, limit of quantification and sensitivity

Limit of detection that obtained for this method was 2.93 mg/g and limit of quantification was 9.75 mg/g. DBS samples with concentrations of more than 1.2 mg/L can be diluted up into the measurement range, while samples with lower concentrations than the detection limit can be concentrated.

The result for the sensitivity showed that the determination of the molar extency value (ϵ) was $2.44 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$. The result indicated that the sensitivity of the method was quite high according to Savin (1979) who stated that the sensitivity of a method was categorized as high if it has a value of molar extency (ϵ) $> 6 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$.

3.1.3 Precision

The analysis showed the analytical method has a good repeatability. This is in accordance with the provisions Horwitz function and AOAC which stated that analyte concentrations below 1 mg/L have precision values received respectively by $<16\%$ and $<11\%$.

Table 1. Intraday precision

[DBS] (mg/L)	Absorbance	SD	% RSD
0.1	0.232	0.0032	1.38
0.6	0.475	0.0323	6.80
1.2	0.823	0.0011	0.14

Table 2. Interday precision

[DBS] (mg/L)	Absorbance	SD	% RSD
0.1	0.233	0.0026	1.13
0.6	0.483	0.0199	4.12
1.2	0.818	0.0040	0.49

3.1.4 Accuracy

Accuracy is expressed as % recovery and determined by spiking method. Spiking process was conducted at the beginning of sample preparation in order to find out that the process of sample preparation involving freezer, freeze dryer, Soxhlet and also evaporator; do not damage DBS surfactant contained in the sample catfish. Table 4 shows the % recovery in the range of 82.63 to 110.60%. The value of % recovery was acceptable because it corresponds to the analyte concentration level as proposed by Gonzalez et al. (2010).

Table 3. Accuracy of analytical method

[DBS] added (mg/L)	[DBS] analyzed (mg/L)	%Recovery
0.015	0.200	95.35
0.030	0.214	92.17
0.060	0.240	90.58
0.120	0.285	82.63
0.150	0.352	110.60

3.2 DBS analysis

The analysis performed on all catfish samples was carried out in its initial stage. DBS metabolism in living organisms can form a carboxylic sulfophenyl (SPC) with a negative charge (Leon et al., 2006), which can not be fully analyzed because of the methylene blue was incapable to bind with SPC. The results of DBS analysis on exposed catfish to various concentrations are presented in Table 5.

Table 4. DBS concentration in catfish

DBS in water (mg/L)	Sample	DBS in catfish (mg/Kg)
2.5	K2.5.I	0.9
	K2.5.II	0.8
	K2.5.III	0.9
5	K5.I	1.7
	K 5.II	1.6
	K5.III	1.7
10	K10.I	4.9
	K10.II	7.2
	K10.III	13.5
15	K 15.I	12.7
	K15.II	25.3
	K15.III	16.3

Results showed that the greater the exposure of anionic surfactant concentration, the more surfactant was accumulated in catfish. Santoso (2010) stated that the

accumulation of surfactant DBS in the body of the organisms increases following with the concentrations of exposure to DBS.

The catfish ability to accumulate DBS shown by the value of BCF in Table 6. Other study also showed that the catfish contained a total value of DBS-BCF was quite large, namely 102, 72 and 42 after exposed with DBS 0.126; 0.293 and 0.927 mg/L for 96 hours. In addition, *Fathead minnow* fish contained total DBS 96, 79, and 65 after exposure to DBS 0.126; 0.293 and 0.927 mg/L for 32 days (Versteeg and Rawlings, 2003).

Table 5. BCF value of DBS in catfish

[DBS] (mg/L)		BCF
Water (mg/L)	Catfish (mg/L)	
2.5	1.33	0.53
5	2.99	0.60
10	6.61	0.66
15	12.40	0.83

Table 6. DBS in the market catfish

Sample	DBS (mg/Kg)
B	61
J	26.5
P	21.8
K	44.5
C	8.5

DBS analysis was not only performed on samples catfish that are set in an environment containing DBS but also on samples from traditional markets in Yogyakarta. This analysis was conducted in order to detect the accumulation of DBS in catfish sold in the traditional market. The results showed in Table 7 that the samples of catfish collected from the traditional market contain DBS. This indicated that the catfish sold in the traditional market were bred in a polluted environment with DBS.

4. Conclusion

The parameters of validation methods have high acceptability as linearity ($R^2 = 0.99$), limit of detection (LOD) 2.93 mg/g and limit of quantification (LOQ) 9.75 mg/g, sensitivity $2.44 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$, precision (RSD) 0.14-1.38% and % recovery in a range 82-110 %. The research showed that catfish can accumulate DBS. The results of DBS analysis in catfish with 2.5; 5; 10; 15 mg/L of DBS concentration exposure respectively are 0.87; 1.67; 8.50 dan 18.10 mg/kg, and catfish from traditional markets in a range 8.5-61 mg/kg. The result showed that the method of analysis of DBS anionic surfactant using MB could be applied for catfish samples.

References

- Chitikela S, Dentel SK, and Allen HE. 1995. Modified Method for The Analysis of Anionic Surfactants as Methylene Blue

- Active Substances. *Analyst*, 120:2001-2004. DOI: <https://doi.org/10.1039/an9952002001>
- Gonzalez AG, Herrador MA, Asuero AG. 2010. Intralaboratory Assessment of Method Accuracy (Trueness and Precision) by Using Validation Standars. *Talanta*, 82:1995-1998. DOI: <https://doi.org/10.1016/j.talanta.2010.07.071>
- Harsojo. 2012. Analisis Makanan dan Lingkungan Secara Fisika-Kimia. Pustaka Pelajar. Yogyakarta.
- Jurado E, Fernandez-Serrano M, Nunez-Olea J, Luzon G, and Lechuga M. 2006. Simplified Spectrophotometric Method using Methylene Blue for Determining Anionic Surfactants: Applications to The Study of Primary Biodegradation in Aerobic Screening Test. *Chemosphere*, 65:278-285. DOI: <https://doi.org/10.1016/j.chemosphere.2006.02.044>
- Koga M, Yamamichi Y, Nomoto Y, Irie M, Tanimura T, and Yoshinaga T. 1999. Rapid determination of anionic surfactants by improved spectrophotometric method using <https://doi.org/10.1016/j.talanta.2011.10.034>
- Otzen D. 2011. Protein-Surfactant Interaction: A Tale of Many States. *Biochim. Biophys. Acta*, 1814:562-591. DOI: <https://doi.org/10.1016/j.bbapap.2011.03.003>
- Saez M, Leon VM, Parra AG, Mazo EG. 2000. Extraction and Isolation of Linier Alkylbenzene Sulfonates and Their Intermediate Metabolites from Various Marine Organisms. *J. Chromatogr.*, 889: 99-104. DOI: [https://doi.org/10.1016/S0021-9673\(00\)00204-1](https://doi.org/10.1016/S0021-9673(00)00204-1)
- Santoso L. 2010. Kajian Toksikitas Dan Bioakumulasi Surfaktan Detergen Linier Alkilbenzen Sulfonate (LAS) Pada Udang Galah *Macrobrachium rosenbergi*. Tesis. IPB. Bogor.
- Schmitt MT. 2001. Analysis of Surfactant. Marcel Dekker, Inc. New York.
- methylene blue. *Anal Sci*, 15:563-568. DOI: <https://doi.org/10.2116/analsci.15.563>
- Leon VM, Lopez C, Martin PAL, Prats D, Varo P, Mazo EG. 2006. Removal of Linier Alkylbenzene Sulfonates and Their Degradation Intermediates at Low Temperatures Du-ring Activated Sludge Treatment. *Chemosphere*, 64:1157-1166. DOI: <https://doi.org/10.1016/j.chemosphere.2005.11.045>
- Munoz DA, Saez M, Martin PAL, Parra AG, and Mazo EG. 2004. New Extraction Method for The Analysis of Linier Alkylbenzene Sulfonates in Marine Organisms Pressurized Liquid Extraction Versus Soxhlet Extraction. *J. Chromatogr. A*, 1052:33-38. DOI: <https://doi.org/10.1016/j.chroma.2004.08.014>
- Olkowska E, Polkowska Z, and Namiesnik J. 2012. Analytical Procedures for The Determination of Surfactants in Environmental Samples. *Talanta*, 88:1-13. DOI:
- Tolls J, Haller M, De Graaf I, Thijssen MATC, and Sijm DTHM. 1997. Bioconcentration of LAS: Experimental Determination and Extrapolation to Environmental Mixtures. *Environ. Sci. Technol.*, 31:3426-3431. DOI: <https://doi.org/10.1021/es970100d>
- Traverso-Soto JM, Gozalez-Mazo E, and Lara-Martin PA. 2012. Analysis of Surfactants in Environmental Samples by Chromatographic Techniques. In: de Azevedo Calderon, Leonardo (Ed.), *Chromatography - The Most Versatile Method of Chemical Analysis*. InTech:187-216.
- Versteeg DJ and Rawlings JM. 2003. Bioconcentration and Toxicity of Dodecylbenzene Sulfonate (C12LAS) to Aquatic Organisms Exposed in Experimental Streams, *Arch. Environ. Contam. Toxicol.*, 44:237-246. DOI: <https://doi.org/10.1007/s00244-002-2017-2>

Conflict of interest: Non declare