

## METHOD VALIDATION AND UNCERTAINTY ESTIMATION FOR ANIONIC SURFACTANTS DETERMINATION FROM MUNICIPAL WASTEWATERS

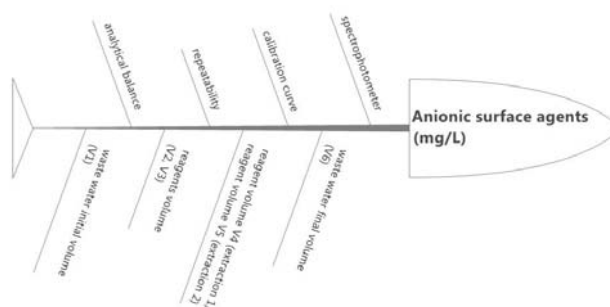
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The analytical method for anionic surfactants measurements in aquatic media was subject of validation procedure. Using two different reference materials the following method performance criteria were investigated: selectivity, linearity domain, precision, accuracy, limit of detection, limit of quantification and robustness. Sources of uncertainties were identified, and extended standard uncertainty was estimated for measurements performed with reference materials solutions. The method was demonstrated to be suitable for tensioactive compounds determination from wastewaters and was further used to determine the anionic detergents content in domestic and industrial wastewaters.



### INTRODUCTION

Surfactants are compounds with molecules having a hydrophobic part such as a nonpolar hydrocarbon chain, and a hydrophilic one, either ionic or non-ionic, but polar. Due to this molecular structure, surfactants tend to organise their molecules based on hydrophilic-hydrophobic interactions, at the interface of two non-miscible different media, acting as tensioactive compounds. The major sources of surfactants in the environment are discharges from household, industrial laundering or other cleaning operation using detergent formulations. Synthetic detergents, commonly called syndets, are anionic, cationic or nonionic surfactants, of which anionic ones are widely used, as sodium sulphates, sulphonates, tripolyphosphates, or silicates. Of these, linear alkylbenzen sulphonates (LAS) are the most used

anionic surfactant in detergents compositions,<sup>1</sup> while polyphosphates undergo fast biodegradation by hydrolysis. Cationic and non-ionic surfactants have higher sorption properties on soils and sediments than anionic surfactants. Most surfactants can be degraded in the environment by microbes, although some surfactants such as LAS, or alkylphenols may be persistent under anaerobic conditions. Even if the hydrolysis products of the surfactants are not acutely toxic to organisms at environmental concentrations and do not pose any threat to aquatic animal life, they act as nutrients for plants and thus cause eutrophication, by excessive plants growth.<sup>2,3</sup>

The studies on the fate and behaviour of the surfactants in different environmental media require analytical methods and techniques that should be valid for these specific measurements. To validate a method means to show that it fits to a

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specific purpose and relies on compulsory demonstration of the following method performance criteria: specificity/selectivity, precision, trueness, linearity and linearity range, limit of detection (LOD), limit of quantification (LOQ), and robustness.<sup>4,5</sup> The international science community is recognizing the need of method validation, not only as quality control mean in the accredited laboratories, but also in the research ones. Table 1 gives some analytical methods applied for anionic surfactants determination, based on validated methods, with the related validation criteria.

The exemplified research studies were mainly using separation methods (chromatography and capillary electrophoresis) applicable for ionic and polar compounds, using different detection systems (fluorescence, UV, MS tandem) to determine anionic, amphoteric or non-ionic surfactants. Even if validation of an analytic method means a carefully examination of methods' characteristics, including robustness,<sup>19</sup> only one study used the robustness performance criteria for method validation. In accredited laboratories, the most applied method for anionic surfactants determination is the one based on spectrometric measurements of the methylene blue active substance (MBAS), which is given in standards like US EPA Method 425.1,<sup>20</sup> EN 903:1993,<sup>21</sup> or SR EN 903-2003.<sup>22</sup>

Valid results are used as bases for decisions, trades, legal actions or publications. The characteristic needed for all kinds of measurements is the so-called measurement uncertainty. Even not yet imposed by all standard methods, measurement uncertainty is a statistical parameter which describes the possible fluctuations of the result of a measurement and can be determined by the addition of the variances of the individual steps of the test procedure.<sup>23</sup> Measurement uncertainty is defined as follows: "Parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand".<sup>24</sup>

The present study gives a validation procedure for anionic surfactants determination method, based on the European/Roumanian standard,<sup>22</sup> using two different standard materials: sodium dodecylsulphate (SDS or sodium lauryl sulphate – C<sub>12</sub>H<sub>25</sub>SO<sub>4</sub>Na) and sodium dodecylbenzene sulphonate (SDBS – C<sub>12</sub>H<sub>25</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>3</sub>Na). The validation criteria were selectivity, calibration curve, LOD, LOQ, precision, accuracy and robustness, according to the EURAGHEM Guide<sup>5</sup>. The sources of uncertainties were identified, and the extended standard uncertainty was estimated for the measurements on wastewater samples.<sup>24</sup> The method is further used to determine the anionic detergents content in domestic and industrial wastewaters.

Table 1

Applications of method validation for anionic surfactants determination from different matrices

	Anionic surfactants	Matrix	Analytical methods	Validation criteria								Ref.
				1	2	3	4	5	6	7	8	
1	linear alkylbenzene sulfonates; sulfophenyl carboxylates	sludge-amended soils	LC-ESI-MS/MS	√			√	√	√	√		6
2	anionic surfactants	river water	FIA - potentiometric	√	√			√	√			7
3	amphoteric surfactants	detergents, shampoos	CE-indirect UV		√	√		√	√	√		8
4	linear alkylbenzene sulfonates	sewage sludge	HPLC-DAD, HPLC-FL	√			√	√	√	√		9
5	linear alkylbenzene sulfonates	sewage sludge	HPLC-DAD, HPLC-FL	√	√	√	√	√	√	√		10
6	linear alkylbenzene sulfonates	sewage sludge	HPLC-FD	√	√	√	√	√	√	√		11
7	nonylphenol/dinonylphenol-poly ethoxylates	process effluents	HPLC-FD	√	√	√	√	√		√		12
8	linear alkylbenzene sulphonates	sewage sludge	HPLC-FD, CE-DAD	√	√	√	√	√	√	√		13
9	16 non-ionic and anionic surfactants	wastewater, river water, seawater	LC-ESI-MS	√	√	√	√	√	√			14
10	perfluoroalkyl phosphonates, carboxylates and sulphonates	drinking water	HPLC-QToF-HRMS	√		√	√	√	√	√		15
11	alcohol sulphates	wastewater	GC-MS	√	√	√	√	√	√	√		16
12	alcohol sulfates and alcohol ethoxysulphates	wastewater samples	LC-ESI-MS	√	√	√	√	√	√	√		17
13	aminopolycarboxylic acids*	river water	GC-MS	√	√	√	√	√	√	√	√	18

where: 1 – selectivity/matrix effect; 2 – repeatability; 3 – interim precision / reproducibility; 4 – accuracy / recovery; 5 – linearity; 6 – LOD; 7 – LOQ; 8 – robustness; CE-DAD – capillary electrophoresis with UV diode array detection; FIA – flow injection analysis; GC-MS – gas chromatography with mass spectrometry; HPLC-FD – high performance liquid chromatography with fluorescence detection; HPLC-QToF-HRMS – high performance liquid chromatography with quadrupole time-of-flight high resolution mass spectrometry; LC-ELSD – liquid chromatography with evaporative light-scattering detector; LC-ESI-MS – liquid chromatography with electrospray ionization mass spectrometry; \*also uncertainty estimation.

## RESULTS AND DISCUSSION

Evaluating the selectivity of MBAS method, Table 2 shows that in the presence of interfering reagents (IR) the concentrations of the RM solutions were significantly different from the ones in the absence of the IR, with differences ( $\Delta\%$ ) ranging between 14.58% up to 39.19%. Lower differences were registered when the SDS RM was used. The results impose that these interfering compounds should be removed from the wastewater samples before measurements.

Calibration curve, limits of detection and limits of quantification were carried out with the solutions of the two chosen RM, correlation coefficient as well as LOD and LOQ were calculated, based on the data registered on the calibration curve (Table 3).

The results show very good correlation coefficients. The standard method<sup>22</sup> requires an

applicability concentration domain of 0.1–5 mg/L, while the estimated LOD is 0.05 mg/L. For both tested RM, LOD and LOQ values are situated between the blank measurement and the first value measured with each RM (for SDBS  $0.004 < 0.012 < 0.020 < 0.096$  and for SDS  $0.002 < 0.005 < 0.014 < 0.177$ ). Moreover, LOD and LOQ are lower than the lower limit of the accepted concentration interval (0.1 mg/L). MBAS method is more sensitive when SDS is used as RM, registering lower LOD and LOQ than those obtained with SDBS.

Table 4 shows the MBAS method precision, tested by mean of repeatability ( $RSD_r$ ) and interim precision ( $RSD_{ip}$ ). From the evaluation data, MBAS method is more precise when using SDBS as reference material, registering lower RDS values for both repeatability and interim precision.

Table 2

Selectivity of the MBAS method for anionic surfactants measurements

IR	$C_{SDBS}$ without IR (mg/L)	$C_{SDBS}$ in the presence of IR		$C_{SDS}$ without IR (mg/L)	$C_{SDS}$ in the presence of IR	
		(mg/L)	$\Delta$ (%)		(mg/L)	$\Delta$ (%)
phenol	0.45	0.59	23.73	0.41	0.52	21.15
potassium cyanide		0.74	39.19		0.34	-20.59
potassium nitrate		0.68	33.82		0.48	14.58

Table 3

Correlation coefficient, LOD and LOQ of the MBAS method for anionic surfactants measurements

RM	$R^2$	Blank value (nm)	First RM measurement (nm)	LOD		LOQ	
				(nm)	(mg/L)	(nm)	(mg/L)
SDBS	0.9997	0.004	0.096	0.012	0.059	0.020	0.099
SDS	0.9991	0.002	0.177	0.005	0.016	0.014	0.037

Table 4

Repeatability and interim precision of the MBAS method for anionic surfactants measurements

Precision	RSD (%)		$C_{RM} \pm$ trust interval (mg/L) (t=2)	
	$RSD_r$ (n=10)	$RSD_{ip}$ (n=15)	$RSD_r$ (n=10)	$RSD_{ip}$ (n=15)
SDBS	0.4386	0.5802	$0.661 \pm 0.0018$	$0.662 \pm 0.0020$
SDS	1.8835	2.5667	$0.410 \pm 0.0048$	$0.420 \pm 0.0056$

Table 5

Accuracy by recovery tests for the MBAS method for anionic surfactants measurements

V (mL)	$C_I$ (mg/L)		$C_A$ (mg/L)		$C_F$ (mg/L)		Recovery (%)	
	SDBS	SDS	SDBS	SDS	SDBS	SDS	SDBS	SDS
1	0.42	0.58	0.11	0.21	0.523	0.77	93.94	90.48
2	0.42	0.58	0.22	0.42	0.633	0.95	96.97	88.89
3	0.42	0.58	0.33	0.63	0.74	1.15	96.97	90.48

Table 6

Robustness evaluation of the MBAS method for anionic surfactants measurements

Robustness	n	RSD (%)		$C_{RM} \pm$ trust interval (mg/L) ( $t=2$ )	
		SDBS	SDS	SDBS	SDS
MB neutral	5	10.404	19.378	$0.464 \pm 0.044$	$0.360 \pm 0.062$
MB acidic	5	10.371	15.002	$0.469 \pm 0.044$	$0.380 \pm 0.051$
reaction time	3x2	0.480	1.239	$0.449 \pm 0.004$	$0.420 \pm 0.002$

Accuracy was also tested for MBAS method, by recovery tests (R%). Using both RMs, the recovery included in the required interval ( $85\% < R\% < 105\%$ ) showing that the method is accurate (Table 5), but the recovery values were closer to 100% when SDBS was used as reference material.

Robustness was tested against two different working conditions that might influence the MBAS method: (i) methylene blue (MB) reagent in neutral or acidic solution; (ii) reaction time. The results are given in Table 6 and revealed that the MBAS method is not robust against the volume of the MB reagent added, but is robust against the reaction time, lower RSD values being registered when SDS was used as RM.

In order to obtain an estimate of the uncertainty associated with a measurement result the following tasks were need to be performed: (i) to specify the measurand; (ii) to identify the sources of

uncertainty; (iii) to calculate the uncertainty components associated with each potential source of uncertainty identified (expressed as standard deviations); (iv) to calculate the combined uncertainty, applying the appropriate coverage factor, to give an expanded uncertainty. The measurand was surfactants content in wastewaters, expressed in mg/L, and the following sources of uncertainty were identified: analytical balance, repeatability, calibration curve, equipment, glassware of different volumes ( $V_1, V_2, V_3, V_4, V_5, V_6$ ), as presented in Fig. 1. Based on the standard uncertainty ( $u_x$ ), and on the relative standard uncertainty ( $u_r$ ), the combined relative standard uncertainty ( $u_c$ ) and expanded standard uncertainty (U) were calculated for surfactants content determination in wastewater samples with SDBS and SDS used as reference materials, and the results are presented in Table 7.

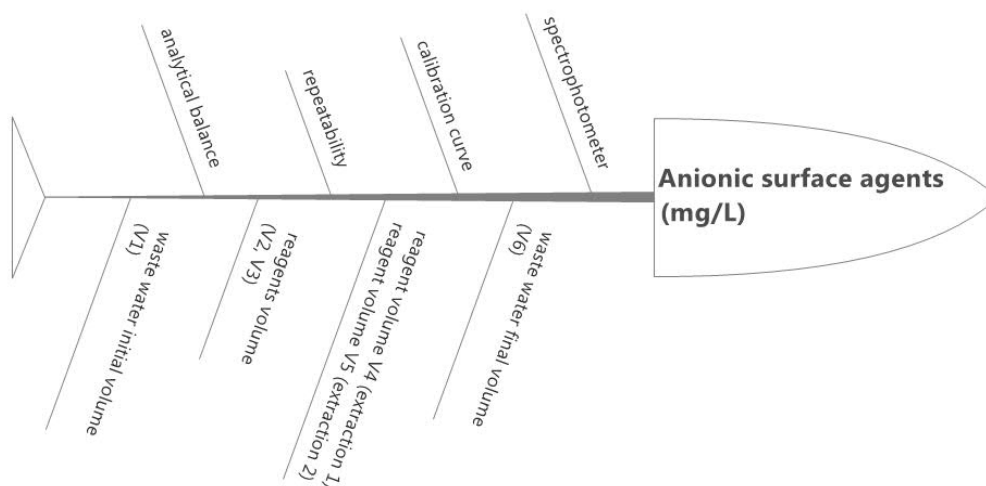


Fig. 1 – Identified sources of uncertainty.

Table 7

Combined relative standard uncertainty and expanded standard uncertainty ( $k = 2, P = 95\%$ )

RM	$u_c$	$u_c\%$	U	U%	$C_{RM} \pm U$ (mg/L)
SDBS	0.0354	3.54	0.0707	7.07	$45.07 \pm 0.0707$
SDS	0.0652	6.52	0.1303	13.03	$51.28 \pm 0.1303$

Table 8

Differences in the behavior of the reference materials used for the validation procedure and uncertainty estimation

	Selectivity	Sensitivity	Precision	Recovery	Robustness	Uncertainty
SDS	√	√			√	
SDBS			√	√		√

The higher contribution to the combined relative standard uncertainty was given by repeatability ( $u_{c,SDBS} = 0.0274$ ,  $u_{c,SDS} = 0.0215$ ), while the smaller contribution was given by the equipment ( $u_{c,SDBS} = u_{c,SDS} = 0.00001$ ).

As expected, the expanded standard uncertainties (U%) are higher than repeatability and interim precision. Lower values of U were registered when the SDBS was used as reference material, similar with the precision results, showing that the method is more trustful when SDBS reference material is used for validation.

The validation criteria as well as uncertainty estimation showed possible preferences in using one of the reference materials or the other one (SDS/SDBS), as presented in Table 8. In terms of selectivity, sensitivity and robustness might be preferred the SDS, while considering precision, accuracy and uncertainty SDBS is more suitable.

## EXPERIMENTAL

### Reagents and method

All the reagents were of analytical grade and two different reference materials (RM) as anionic surfactants were tested: sodium dodecylbenzene sulphonate (SDBS), purchased from Acros Organics (with 88% purity) and sodium

dodecylsulphate (SDS), purchased from Merck (with 99.0 purity). The used glassware (volumetric flasks and pipettes) were of A class. Measurements were carried out with a UV-VIS spectrophotometer (Secomam 750 I, 1222 series (applicability domain  $\lambda = 195-1100$  nm). The validation procedure was performed according to the Roumanian standard,<sup>22</sup> based on spectrometric measurements of the methylene blue index, MBAS.

Selectivity of the MBAS method was evaluated comparing the concentration of the RM solution in the absence and in the presence of the interfering reagents (phenol, potassium cyanide and potassium nitrate), as specified in the Romanian standard,<sup>22</sup> Calibration curves were determined with RM solutions of 6 different concentrations. LOD and LOQ were calculated based on equations 1-3, for 10 blank measurements. Precision was evaluated by repeatability ( $n=10$ ) and interim precision (5 replicates measured in 3 different days), based on relative standard deviations (RSD%) calculated using equations 3-4, and the result was expressed also considering the confidence interval, calculated with equations 5-6. Accuracy was evaluated based on recovery test, using equation 7, applied for measurements of three replicates, for each sample solution volumes. Robustness was tested against two different working conditions that might influence the method: (i) methylene blue (MB) in neutral or acidic solution, different volumes of MB solution were used (1, 3, 5, 7 and 9 mL, 5 mL being the volume required by the standard); (ii) reaction time (measured immediately and after 30 minutes). Robustness evaluation was based on RSD parameter. Uncertainty was estimated based on calculations of standard uncertainty ( $u_x$ ), relative standard uncertainty ( $u_r$ ), combined relative standard uncertainty ( $u_c$ ) and expanded standard uncertainty (U), according to equations 8-11.

### Used formulae

$$x_{LOD} = \bar{x}_{blank} + 3 s_{blank} \quad (1)$$

$$x_{LOQ} = \bar{x}_{blank} + 10 s_{blank} \quad (2)$$

$$s = \sqrt{\frac{\sum_{i=1}^n (\bar{X} - X_i)^2}{n-1}} \quad (3)$$

$$RSD = \frac{s}{\bar{x}} \cdot 100 \quad (4)$$

$$\bar{x} \pm t \cdot s_x \quad (5)$$

$$s_x = \frac{s}{\sqrt{n}} \quad (6)$$

$$R = \frac{C_F - C_I}{C_A} \cdot 100 \quad (7)$$

$$u_x = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n-1}} \quad (8)$$

$$u_r = \frac{u_x}{x} \quad (9)$$

$$u_c = \sqrt{\sum u_r^2} \quad (10)$$

$$U = u_c \cdot k \cdot 100 \quad (11)$$

where:  $x_{LOD}$  – limit of detection;  $\bar{X}_{blank}$  – average value registered with the blank solution;  $s_{blank}$  – standard deviation of the measurements with blank solution;  $x_{LOQ}$  – limit of quantification;  $s$  – standard deviation;  $n$  – number of discrete measurements;  $\bar{X}$  – average value of  $n$  repeated measurements;  $X_i$  – discrete measured values; RSD – relative standard deviation;  $s_x$  – standard deviation of the mean;  $t$  – normal distribution factor;  $R$  – recovery;  $C_F$  – final concentration;  $C_I$  – initial concentration;  $C_A$  – added concentration;  $u_x$  – standard uncertainty;  $u_r$  – relative standard uncertainty,  $u_c$  – combined relative standard uncertainty;  $U$  – expanded standard uncertainty.

## CONCLUSIONS

The analytical method for anionic surfactants measurements in aquatic media, was validated. The method is selective, sensitive, precise and accurate. It was demonstrated that the method is not robust against the volume of the methylene blue solution, but is robust against the reaction time. Differences in the evaluation results were registered for the two reference materials used for validation procedure, sodium dodecylsulphate and sodium dodecylbenzene sulphonate. Based on the identified sources of uncertainty, different types of uncertainties were estimated for measurements on wastewater samples.

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