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Analytical validation of a modified turbidimetric assay to screen sulphur oxidizing bacteria

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ABSTRACT

Conventional turbidimetric assay for sulphate determination was modified to 100 times lesser reaction volume on a convenient format using microtitre plate based platform, targeting routine microbiological applications to screen sulphur oxidizing bacteria (SOB) cultures. The modified assay was linear up to 1500 mg/L of sulphate concentration, which is about 37.5 times more than that of conventional assay. Upon regression analysis, linear equation y = 1.243× + 0.011 was obtained having R^2 value of 0.998. The modified assay was fully validated in terms of precision, limit of detection (LOD), limit of quantification (LOQ), sensitivity, selectivity and robustness to assure the reliability during final applications. LOD and LOQ were found as 7.4 mg/L and 24.8 mg/L of sulphate concentration respectively. Further, accuracy of the assay over routine SOB screening media components was tested, and proved as reliable and suitable for the intended application.

1. Introduction

Increasing anthropogenic activities like petroleum refining (Tang et al., 2009), pulp and paper manufacturing (Janssen et al., 2009), food processing, livestock farming cum aquaculture (Chung et al., 2001), natural gas processing (Tang et al., 2009) and mining (Vera et al., 2013) have resulted in the discharge of considerable quantities of sulfide and other reduced sulphur compounds in waters and gas streams. Reduced sulphur compounds prevailing in these industrial, municipal and agriculture waste contributes to one of the most troublesome issues in environment (Lin et al., 2018). Higher accumulations of these compounds create an imbalance on environmental sulphur cycle, leading to several issues worldwide, such as odor nuisance, corrosion, sulfide toxicity and acid rain (Lens and Kuenen, 2001; Pikaar et al., 2014). Consequently, various technologies and strategies have been developed and used to deal with different reduced sulphur compounds (Sercu et al., 2005). Among these techniques, biological oxidation accomplished through sulphur oxidizing bacteria (SOB) is gaining more attraction, due to its low operational requirements, flexibility, safety and easiness (Buisman et al., 1989; Sercu et al., 2005). This emerging interest on SOB's application has led to fine exploration of diverse ecological niche for potential strains (Pokorna and Zabranska, 2015).

Screening methodologies for SOB stems on analytical techniques for quantification of their final metabolite namely, sulphate through gravimetric, volumetric and turbidimetric methods, ion selective electrode, ionic chromatography, capillary electrophoresis and analysis by flow injection (Meneses et al., 2005; Fung et al., 2008; Caceres et al., 2015). Even though, each of these techniques has its own merits, they require special equipment, qualified personnel, high operational cost, and are tedious, unless automated (Fung et al., 2008; Caceres et al., 2015). Whereas, turbidimetric technique based on sulphate precipitation with barium chloride, is considered as the most reliable method for microbiological applications due to its simplicity and low requirements for instrumentation (Meneses et al., 2005; Severiche and Gonzalez, 2012). However, the conventional turbidimetric method is based on large sample volume (> 50 mL) and fairly time consuming (> 1 h) (APHA, 1975). Further, the accuracy for this method is limited to 40 mg/L sulphate concentration (APHA, 1998). Even though conventional turbidimetric assay has been applied for SOB screening by various authors (Asano et al., 2007; Behera et al., 2014, 2016; Huber et al., 2016), analytical validation in culture conditions has not been conducted till date. Considering all these facts, the present study targets to modify the conventional APHA assay in a lesser volume, convenient format with minimal time on microtitre plate platform targeting routine microbiological applications. Reliability of the modifications was then validated through recommended guidelines (ICH, 1995; ICH, 2005). Further, the assay was reciprocated in routine SOB media to assure the accuracy and selectivity over media components.

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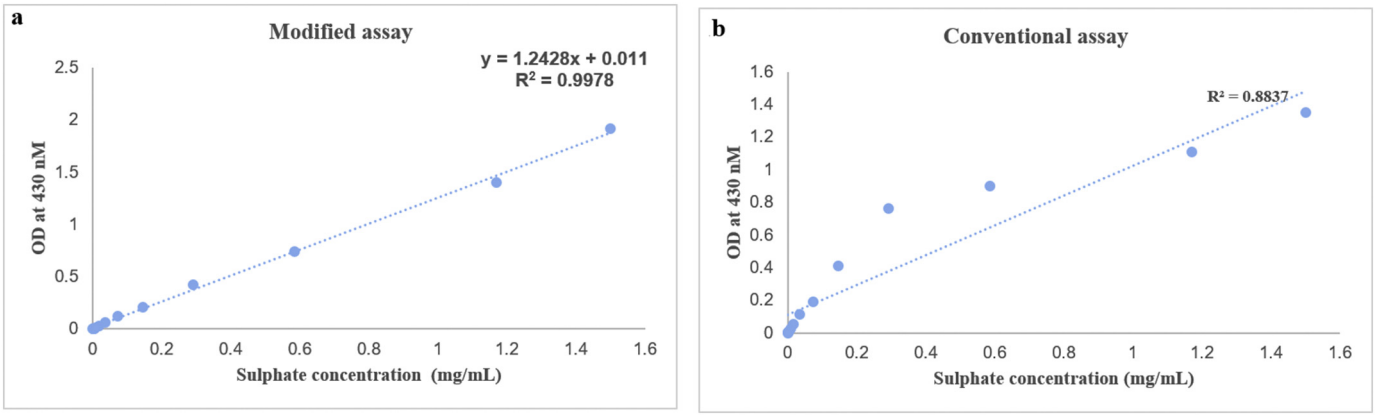

Fig. 1. Calibration curves at higher sulphate concentrations of modified assay (a) and conventional assay (b).

Table 1
Primary parameters in modified analytical assay validation.

Sulphate concentration (mg/L)	Mean absorbance	SD	RSD (%)	95% confidence intervals for mean	
				Lower bound	Upper bound
1500	1.91433	0.00687	0.36	1.90977	1.91889
1171	1.4	0.00913	0.65	1.39394	1.40606
585	0.739	0.0125	1.68	0.73074	0.74726
292	0.42483	0.00321	0.76	0.42270	0.42696
146	0.20475	0.00388	1.89	0.20218	0.20732
73	0.12483	0.00195	1.56	0.12354	0.12613
36	0.06536	0.00125	1.91	0.06453	0.06619
18	0.0271	0.00053	1.97	0.02675	0.02745
9	0.00944	0.00019	2.00	0.00931	0.00956
4.5	0.00514	0.00032	6.12	0.00493	0.00535
3.5	0.00161	0.00021	12.88	0.00148	0.00175
2.25	0.00038	0.00007	19.17	0.00033	0.00043
1	0.00006	0.00002	27.76	0.00005	0.00007

2. Materials and methods

2.1. Modified turbidimetric assay protocol

Modification was done in conventional turbidimetric assay which was based on precipitation of sulfate ions with barium chloride (APHA, 1975). The targeted modifications included minimizing the reaction volume, convenient assay platform and reagent forms. The modified assay protocol was as follows; 500 µL of solution containing sodium sulphate (ranging concentrations of 1–40 mg/L) was added to 25 µL of conditioning agent (composition: 15 g sodium chloride, 10 mL glycerol, 6 mL concentrated hydrochloric acid and 20 mL of 95% ethanol which was made up to 100 mL using milliQ water) in 1.5 mL centrifuge tube. An equal volume (525 µL) of 10% barium chloride solution was added to this reaction mixture. After incubation of 5 min, 300 µL of the reaction mixture was aliquoted to a microtitre plate in triplicates and absorbance was measured at 430 nm. The reaction mix devoid of sodium sulfate was used as blank. The assay was carried out in triplicates and, mean value of the absorbance was subtracted from blank and plotted against sulphate concentration of each solution; which were then fitted to a straight line using linear regression analysis (Barwick, 2003). Meanwhile, conventional turbidimetric method was carried out using the same standard solutions (APHA, 1975). In the final step, 300 µL of the reaction mixture of various standard solutions was aliquoted to a microtitre plate in triplicates and absorbance was measured at 430 nm concurrently. The standard curves prepared using both assays (modified and conventional assays) were compared using standard analytical terminologies (Belouafa et al., 2017).

2.2. Modified assay in higher sulphate concentrations

To redefine the linear range in which analyte concentration can be measured accurately, the modified assay was conducted in standard sulphate solutions beyond the recommended range of conventional turbidimetric assay (1–40 mg/L). For this, standard sulphate solutions were prepared by dissolving sodium sulphate in different concentrations ranging from 0 to 150 g/L milliQ water and were subjected to the modified assay protocol. Mean absorbance values were plotted against sulphate concentration and a standard curve was prepared. The curve was then compared to the standard curve prepared using absorbance of the same solutions obtained through conventional turbidimetric assay as described previously.

2.3. Analytical validation of the modified assay

Modified assay was then subjected to analytical validations as per International Conference on Harmonization guidelines (ICH, 1995, 2005). Linear regression analysis was used to evaluate the linearity of calibration curve by the least square linear regression method. Primary parameters required in result interpretation of assay validation namely, mean, standard deviation, relative standard deviation and confidence intervals (Belouafa et al., 2017) of absorbance values were then calculated for each sulphate concentration. Assay precision was examined by performing 12 individual analyses of samples containing varied sulphate concentrations within the assigned linear range (Ryder and Clarke, 2002). Limit of Detection (LOD) and LOQ (Limit of Quantification) were subsequently calculated using the formulae (3*SD)/m and (10*SD)/m respectively, where 'SD' denoted standard deviation of response and 'm' denoted slope of calibration curve (Shrivastava and Gupta, 2011).

2.4. Validation of the modified assay in SOB media conditions

Robustness of the method under different pH conditions, the parameter getting changed in media by the growth of SOB (Behera et al., 2014) was then checked by making deliberate pH variations (1, 2, 3, 4, 7, 9) in reaction conditions (ICH, 2005). Selectivity coefficient of the assay on sulphate over thiosulphate which is usually present in routine SOB media (Huber et al., 2016) was subsequently determined by conducting the assay on varied sulphate concentrations, either in the presence or in the absence of thiosulphate (Valcarcel et al., 2001). Further, dilution factor required prior to assay, for routinely used SOB media was estimated in order to delimit the accuracy of modified assay within acceptable limits. For this, varied concentrations of sulphate was spiked in undiluted and various dilutions of two routinely used SOB media (Teske et al., 2000; Behera et al., 2016). Recovery % of sulphate and RSD (relative standard deviation) of absorbance values in each dilutions

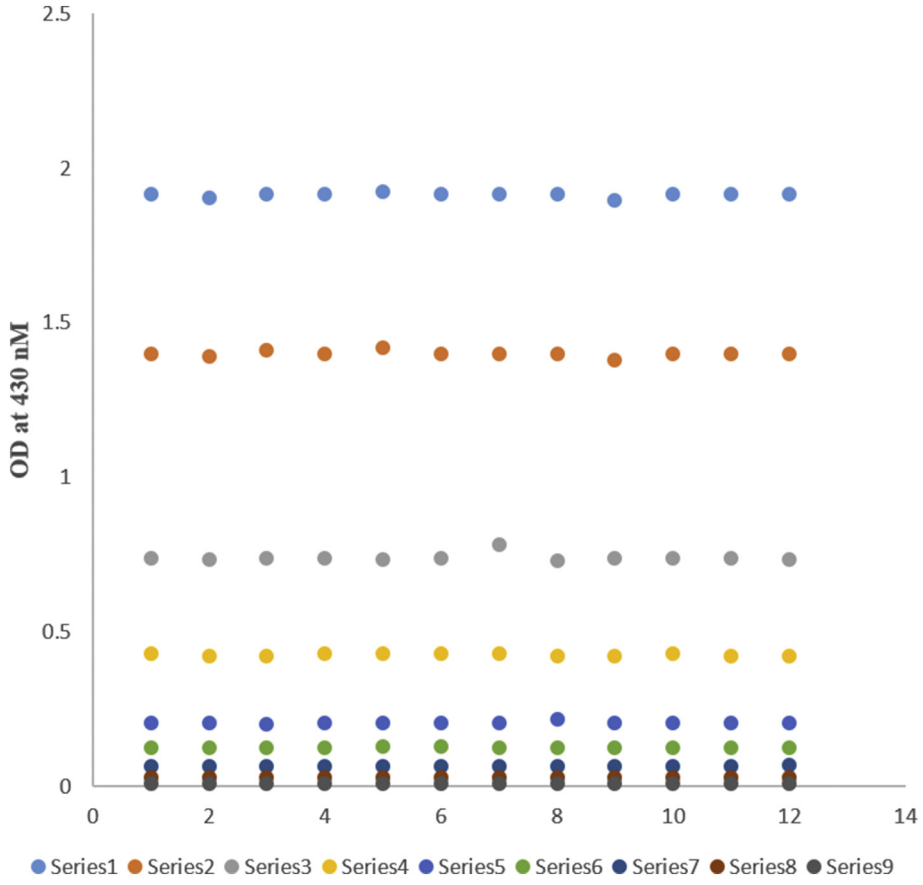

Fig. 2. Results of assay precision.
Each series indicates sulphate concentration in mg/mL as follows; Series 1: 1.5; Series 2: 1.171; Series 3: 0.585; Series 4: 0.292; Series 5: 0.146; Series 6: 0.073; Series 7:0.036; Series 8: 0.018; Series 9: 0.009

of media were calculated (Gonzalez and Herrador, 2007).

2.5. Statistical analysis of the results

Statistical analysis of the assay validation was conducted as per Belouafa et al. (2017). Significance of linear regression model was checked by one-way ANOVA test with p value < .05 set to represent significant difference. One-way ANOVA was also used to compare the absorbance means between different sulphate concentrations and between varied pH conditions with p value < .05 set to represent the significant difference. After one way ANOVA, Tukey's test was used for post-hoc analysis. All statistical analyses were conducted using SPSS software program ver. 16.

3. Results and discussion

Validation of analytical method prior to wider applications, is essential to prove its accuracy and reliability within the scope of its intended use (Bridwell et al., 2010). Understanding the applications and limitations of a test though assay validation will permit an accurate and precise sample assessment (ICH, 1995). Here, we modified the conventional turbidimetric assay for determining sulphate concentration so that, it can be used for routine and simultaneous screening of many SOB cultures with minimal time and reaction volume in a convenient assay platform and reagent forms. The modified assay was then subjected to the recommended analytical validations (ICH, 1995; ICH, 2005) to ensure the accuracy and precision of outputs. Further, the assay was reciprocated in routine SOB media to assure the accuracy and selectivity over media components.

Conventional turbidimetric method is based on precipitation of sulphate ions with barium chloride under controlled conditions (APHA, 1975). The resulting turbidity is determined by a nephelometer or a spectrophotometer, as the turbidity formation is directly proportional to sulphate concentration. SOB which are having capability to oxidize reduced sulphur compounds, form sulphate as the final product, so that increasing sulphate concentration in screening media can be used as indication for the presence/potential of SOB (Chaudhary et al., 2019). Even though conventional turbidimetric assay has been applied in SOB screening methodologies by various authors (Asano et al., 2007; Behera et al., 2014, 2016; Huber et al., 2016), analytical validation of the assay in culture conditions has not been conducted till date. Further, the final reaction volume of conventional method is 105 mL. To ensure the convenience, easiness and economic feasibility in its application for routine microbiological purposes, a modification was attempted by reducing the total reaction volume to 100 times. The use of barium chloride solution instead of barium chloride powder was another modification, to yield a convenient and reproducible platform. Further, simultaneous screening of multiple cultures with high degree of reproducibility was ensured using microtitre plate (Walkowiak et al., 1997).

Modified assay was initially compared with the conventional assay to validate its performance within the recommended range of detection (1–40 mg/L), using sodium sulphate as standard. From regression analysis of the standard curves, linear equation was obtained as; $y = 1.8911 \times -0.0045$ and $y = 3.256 \times -0.0016$, with coefficient of determination (R^2) as 0.992 and 0.997 for modified and conventional assay respectively. Thus, both assays indicated a strong linear relationship between sulphate concentration and absorbance values within the detection range. ANOVA analysis for both assays (Supplementary Tables 1, 2) proved that the regression model can

Table 2
Results of robustness in modified assay.

Sulphate concentration (mg/L)	pH	Mean absorbance	SD	RSD (%)	95% confidence intervals for mean		Over all sig value
					Lower bound	Upper bound	
1500	1	1.899	0.018	0.97	1.88	1.918	0.074
	2	1.901	0.011	0.57	1.88	1.918	
	3	1.904	0.014	0.76	1.889	1.912	
	4	1.917	0.002	0.11	1.889	1.920	
	7	1.915	0.006	0.33	1.915	1.919	
	9	1.917	0.005	0.25	1.909	1.922	
1171	1	1.396	0.018	1.27	1.378	1.415	0.670
	2	1.392	0.018	1.32	1.372	1.411	
	3	1.392	0.025	1.78	1.366	1.418	
	4	1.393	0.011	0.79	1.381	1.404	
	7	1.402	0.012	0.83	1.389	1.414	
	9	1.406	0.007	0.53	1.398	1.414	
585	1	0.716	0.012	1.63	0.704	0.729	0.933
	2	0.709	0.012	1.63	0.697	0.721	
	3	0.718	0.013	1.82	0.704	0.732	
	4	0.746	0.015	1.98	0.731	0.762	
	7	0.74	0.01	1.35	0.729	0.750	
	9	0.744	0.014	1. 88	0.729	0.758	
292	1	0.417	0.005	1.26	0.411	0.422	0.101
	2	0.42	0.005	1.23	0.414	0.425	
	3	0.421	0.004	0.84	0.417	0.425	
	4	0.424	0.004	0.88	0.420	0.428	
	7	0.421	0.005	1.22	0.415	0.426	
	9	0.422	0.004	1.00	0.418	0.427	
146	1	0.21	0.004	1.93	0.206	0.214	0.222
	2	0.208	0.004	1.84	0.204	0.217	
	3	0.203	0.003	1.66	0.199	0.206	
	4	0.210	0.004	1.84	0.206	0.214	
	7	0.213	0.004	1.92	0.208	0.217	
	9	0.208	0.004	1.92	0.204	0.212	
73	1	0.122	0.002	1.98	0.12	0.125	0.405
	2	0.121	0.002	1.98	0.119	0.124	
	3	0.121	0.002	1.88	0.119	0.123	
	4	0.123	0.002	1.83	0.121	0.126	
	7	0.123	0.002	1.23	0.122	0.125	
	9	0.123	0.002	1.99	0.121	0.126	
36	1	0.061	0.001	1.76	0.06	0.062	0.511
	2	0.061	0.001	1.83	0.059	0.062	
	3	0.061	0.001	1.78	0.059	0.062	
	4	0.061	0.001	1.97	0.06	0.062	
	7	0.061	0.001	2.00	0.060	0.063	
	9	0.062	0.001	1.96	0.061	0.063	
18	1	0.026	0.001	1.92	0.026	0.027	0.413
	2	0.026	0.001	1.96	0.026	0.027	
	3	0.026	0.001	1.43	0.026	0.027	
	4	0.027	0.001	2.00	0.026	0.027	
	7	0.027	0.001	2.00	0.026	0.027	
	9	0.026	0.001	2.00	0.026	0.027	
9	1	0.0092	0.0001	1.99	0.0090	0.0094	0.184
	2	0.0092	0.0001	1.46	0.0089	0.0093	
	3	0.0091	0.0001	1.61	0.0089	0.0093	
	4	0.0093	0.0002	1.88	0.0092	0.0095	
	7	0.0090	0.0002	1.92	0.0091	0.0095	
	9	0.0093	0.0002	1.93	0.0091	0.0095	

predict the outcome variable significantly for both assays within the recommended range ($p < .05$).

In order to define the linear range upon modification, solutions containing higher sulphate concentrations (0 to 150 g/L) were subsequently used as the substrates for the modified assay. When mean absorbance values were plotted against sulphate concentration, the linearity was found to be maintained up to 1500 mg/L. Regression analysis for the same indicated a strong linear relationship and a very good representation of the data by regression equation (Fig. 1a) ($y = 1.243\times + 0.011$, $R^2 = 0.998$, $p < .05$) (Supplementary Table 3). Corresponding R^2 value of conventional assay within 0 to

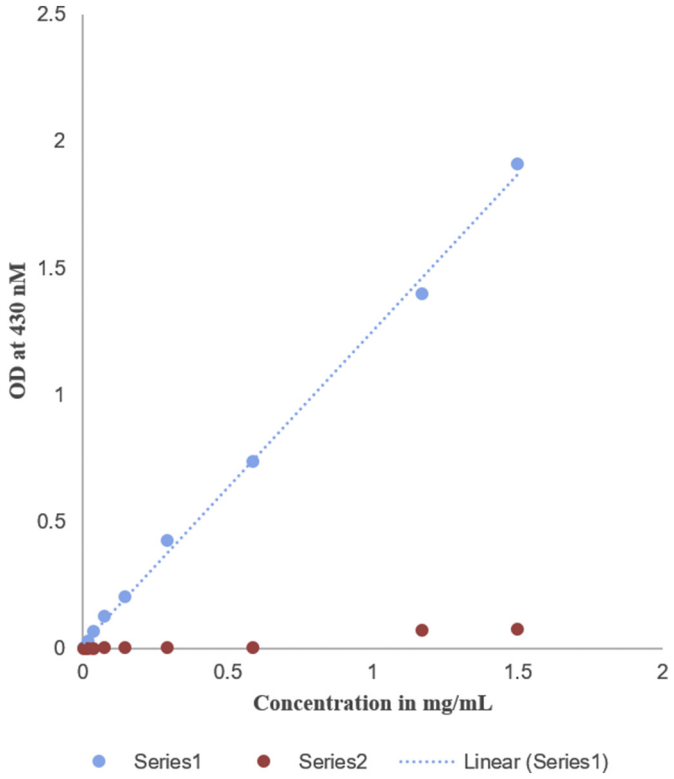

Fig. 3. Selectivity of the modified assay for sulphate over thiosulphate. Series 1: Sulphate; Series 2: Thiosulphate.

1500 mg/L sulphate concentration was only 0.8837 (Fig. 1b), indicating superiority and applicability of modified assay over the conventional assay. Another major drawback of conventional assay was its inability to screen multiple samples simultaneously in microtitre plate, due to the interference by the turbidity upon the increased amount of precipitation. Use of minimal reagents and time in modified assay could resolve the issue, so that simultaneous screening was made possible.

Primary parameters required in the result interpretation of analytical assay validation namely, mean, standard deviation, relative standard deviation (RSD) and confidence intervals (Belouafa et al., 2017) corresponding to each sulphate concentration within linear range of the modified assay were given in Table 1. Limit of Detection (LOD) and Limit of Quantification (LOQ) were found as 7.4 mg/L and 24.8 mg/L respectively. Sensitivity of the modified assay above the LOD was 1.41 ± 0.24 mg/L. One way ANOVA with Tukey post-hoc analysis revealed that there was an overall significant difference in absorbance values between different sulphate concentration ($p < .05$). Further, there is a statistically significant difference in absorbance values among different concentrations above LOD ($p < .05$). However, there were no significant differences in absorbance values among concentrations below LOD ($p > .05$). More importantly, reliable reading of modified assay was up to 1500 mg/L, which was about 37.5 times more than the conventional assay (40 mg/L, APHA, 1975). This increment in upper range of the assay is very useful in SOB screening methodology as the concentration of the substrate for SOB activity (eg. thiosulphates) in the media is in the range of 1000 to 1500 mg/L. Hence, there is every possibility that a potential SOB can utilize a high amount of this substrate and eventually convert to sulphate. Quantitative information of such potential microbes might not be accurate by conventional assay, even after the absorbance of media has been equated to that of equivalent to milliQ water, prior to quantification.

Precision of a method is “the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions” (Armbruster et al.,

Table 3
Results of accuracy testing on two routinely used SOB media.

Sulphate concentration (mg/L)	Mean absorbance		SD		RSD (%)		Recovery %	
	1[a]	2[a]	1	2	1	2	1	2
1500	1.9456	2.0017	0.0385	0.0271	1.98	1.36	101.57 ± 2.09	104.47 ± 1.5
1171	1.4005	1.4287	0.0076	0.0161	0.54	1.13	100.18 ± 0.43	101.81 ± 1.52
585	0.7377	0.7407	0.0069	0.012	0. 94	1.62	99.63 ± 1.97	100.68 ± 1.61
292	0.4111	0.4898	0.007	0.0059	1.7	1.20	98.11 ± 1.06	115.04 ± 1.75
146	0.2206	0.2132	0.0024	0.0012	1.09	0.55	103.79 ± 1.66	104.92 ± 0.85
73	0.1304	0.1312	0.0018	0.0013	1.39	1.01	104.02 ± 2.40	104.40 ± 1.92
36	0.0638	0.0624	0.0011	0.0006	1.72	1.01	103.92 ± 3.09	95.54 ± 1.47
18	0.0263	0.0243	0.0004	0.0005	1.70	2.00	98.68 ± 2.23	90.18 ± 1.79
9	0.0091	0.0091	0.0002	0.0002	1.99	1.92	96.23 ± 2.49	97.74 ± 4.5

[a] 1 denotes SOB media 1 (Teske et al., 2000) and 2 denotes SOB media 2 (Behera et al., 2016).

1994) and it is normally expressed as relative standard deviation, RSD (Le and Phung, 2019). When precision of the modified assay in different sulphate concentrations within linear range (Fig. 2) was calculated, results showed that RSD values above LOD were ≤ 2%. Thus the results suggested that the method was very precise above LOD, and has capacity to generate reproducible results between independent assays (Ryder and Clarke, 2002; Le et al. 2019).

As the next step, feasibility of the modified assay in SOB media conditions were validated. As growth of SOB will results in pH reduction of culture medium (Behera et al., 2014; Veerender et al., 2014), it is essential that the adopted assay for SOB screening should perform robust in different pH conditions. Thus, robustness of the assay in different pH conditions (1 to 9) was evaluated initially, and results (Table 2) showed that robustness was within the acceptable limits (RSD ≤ 2.0%) (Lee et al. 2019). Further, one-way ANOVA with Tukeys post-hoc test showed no significant difference in absorbance values of varied sulphate concentration under different pH conditions ($p > .05$). The second validation carried out was upon the selectivity of the assay, where selectivity is the measure of freedom from interferences due to the presence of other substances (Valcarcel et al., 2001). Sodium thiosulphate is the usual ingredient at a concentration of 1000 to 1500 mg/L in many routinely used SOB media (Behera et al., 2014; Veerender et al., 2014; Huber et al., 2016). To check the possibility of interference from thiosulphate on absorbance values of sulphate, the assay was conducted on various sulphate concentrations within the linear range, both in the presence and absence of thiosulphate (0 to 1500 mg/L). Selectivity coefficient, the measure of an interferent's potential effect on analysis (Valcarcel et al., 2001) was calculated as 0.006 ± 0.013, which is less than +1; indicates that the method was highly selective for sulphate over thiosulphate within the recommended range (Valcarcel et al., 2001).

Our next aim was to find out the dilution factor required prior to assay, for routinely used SOB media in order to delimit the accuracy of modified assay within acceptable limits; where, accuracy expresses the closeness of results in different sulphate concentration obtained in SOB media to that in milliQ water (Gonzalez and Herrador, 2007). Accuracy of analytical assay is expressed in terms of recovery % and RSD%; and acceptable limits are 80 to 120%, ≤ 2% for recovery and RSD respectively (Tiwari and Tiwari, 2010; Le and Phung, 2019). Two common media, one routinely used for the isolation of autotrophic SOB (Teske et al., 2000) and another, for heterotrophic SOB (Behera et al., 2016) were used to check the accuracy. A minimum dilution of 1: 4 and 1: 125 was found to be required for heterotrophic and autotrophic SOB media respectively, so that average OD value of un-inoculated media (0.101 and 1.97 respectively) got equivalent to that of milliQ water. Dilution of autotrophic SOB media (1: 125) was not unexpected, as un-inoculated autotrophic SOB media itself contains ammonium sulphate and magnesium sulphate (Teske et al., 2000). These initial sulphate will be an add-on to the sulphate formation through SOB metabolism hindering the accurate performance of turbidimetric assay. As the initial sulphate concentration itself is above the linear range, dilution is necessary to delimit the sulphate concentration within the linear range of the assay for having precise quantitative information. Further, necessary dilution of heterotrophic SOB media (1:4) was unexpected, which might be due to the mild non-specific reaction of thiosulphate (present in media @ 1000 mg/L) in the assay (Fig. 3). Results of accuracy testing in appropriate dilution of both SOB media were given in Table 3. It was found that mean recovery rate of sulphate and mean RSD values of absorbance from 1:4 diluted heterotrophic and 1:125 diluted autotrophic medium was 101.6 ± 1.89%, 100.68 ± 1.94%, 1.3 ± 0.47% and 1.45 ± 0.498% respectively, both comes within the accepted limits (Tiwari and Tiwari, 2010; Le and Phung, 2019), suggesting that the assay can be applied reliably in both SOB media. Dilution of samples to fit the analyte concentration into the range of standard curve is a well-accepted criterion for any assay. Through this study, we recommend that dilution of any SOB media has to be carried out in such a manner that the absorbance of un-inoculated media get equivalent to that of milliQ water before performing the assay. This will ensure maximum accuracy on quantitative information upon sulphur oxidization potential, while screening through turbidimetric assays.

4. Conclusions

In conclusion, conventional turbidimetric assay for sulphate concentration was modified in such a way that it can be applied very conveniently for the screening of multiple SOB cultures. Use of minimal reagents in a convenient format lowers the cost and required sample volume for testing (500 µL), offering a help in daily monitoring of suspected cultures. Possibility of using microtitre plate for reading the absorbance will facilitate the simultaneous monitoring of multiple SOB cultures. More importantly, 37.5 fold increment in detecting sulphate concentration was possible when compared to the conventional assay. The dilution factor required prior to assay, for routinely used SOB media in order to delimit the accuracy of modified assay within acceptable limits was also calculated. Concisely, the modified assay was fully validated in terms of linearity, precision, LOQ, LOD, sensitivity, robustness, selectivity and accuracy in SOB media conditions; and was proved as reliable and suitable for the intended application.

Author contributions

APV and AF executed the experimental design and APV combined the observations. STG and RKJ conceived of the presented idea, supervised the findings and wrote the manuscript. VP supervised the project and facilitated the experiments. SS helped in analysis of results.

Data and materials availability

Data associated with this study are available within the article or its supplementary materials.

Author contributions

APV and AF were involved in methodology, validation and visualization of data. STG and RKJ were involved in conceptualization, formal analysis, supervision of experiments and writing the manuscript. VP was involved in funding acquisition and project administration. SS helped in the formal analysis of the results.

Declaration of Competing Interest

Authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mimet.2020.105998.

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