Development and Validation of Analytical Methods for Elemental Sulphur in Alberta Soils

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For Elemental Sulphur in Alberta Soils

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Development and Validation of Analytical Methods for Elemental Sulphur in Alberta Soils

Developed by Maxxam Analytics

for

Alberta Environment and Parks

June 2015

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1 Background

Deposition of air-borne sulphur dust from industrial facilities that handle or process solid elemental sulphur has the potential to cause soil acidification at or near those sites in Alberta. Lack of suitable analytical methods for elemental sulphur (S8) has become a challenge for soil monitoring for industry operators and regulators. An acetone extraction-sodium cyanide colorimetric analysis method (Maynard and Addison, 1985) has been used in Alberta but environmental consultants and laboratories complained about the high detection limit and questioned its reliability. A chloroform extraction-HPLC analysis method developed in New Zealand (Watkinson *et al*., 1987) was reported to work well with a wide range of agricultural mineral soils and some sediments. It was not clear if it would be suitable in Alberta because the impact of elemental sulphur on soils is frequently monitored in forested sites where organic materials from forest litter may interfere. There was also a need to assess if the above-noted methods were compatible with the analytical laboratories in Alberta. As chloroform is a toxic substance, alternative solvents of similar extracting capacity for elemental sulphur needed to be explored.

Maxxam Analytics was previously retained by the former Department of Alberta Environment and Sustainable Resource Development (ESRD) to calibrate and refine the above-noted methods and, if needed, to develop alternative analytical methods and subsequent standard operating procedures (SOP). This project was conducted with practical input from a network of commercial laboratories in Alberta. Maxxam Analytics also coordinated a round robin project, with participating members from the network of commercial laboratories in Alberta. A final project report was submitted to ESRD in May and the round robin report in July of 2013.

This combined report is prepared at the request of Alberta Environment and Parks (AEP) for operational applications and has incorporated elements of the two foregoing documents. The rationale, approaches, and results presented in this report form the basis for the SOP, which promotes consistency in implementation of the analytical method. The SOP is included as Attachment A and the round robin results as Attachment B.

2 Initial Investigations

Preliminary experiments were conducted to assess sample preparation and analysis options. Once completed, the data were compiled and reviewed with ESRD in order to select the optimum procedure for full method validation.

2.1 Standard Integrity

Literature references and our initial investigations showed that elemental sulphur working standards contained some S6 and S7 and perhaps polymeric species, as well as S8. Representative chromatograms of a 200 µg/mL injection of the reference standard are shown in Figures 1a and 1b. Our chromatographic investigations showed that for the primary and

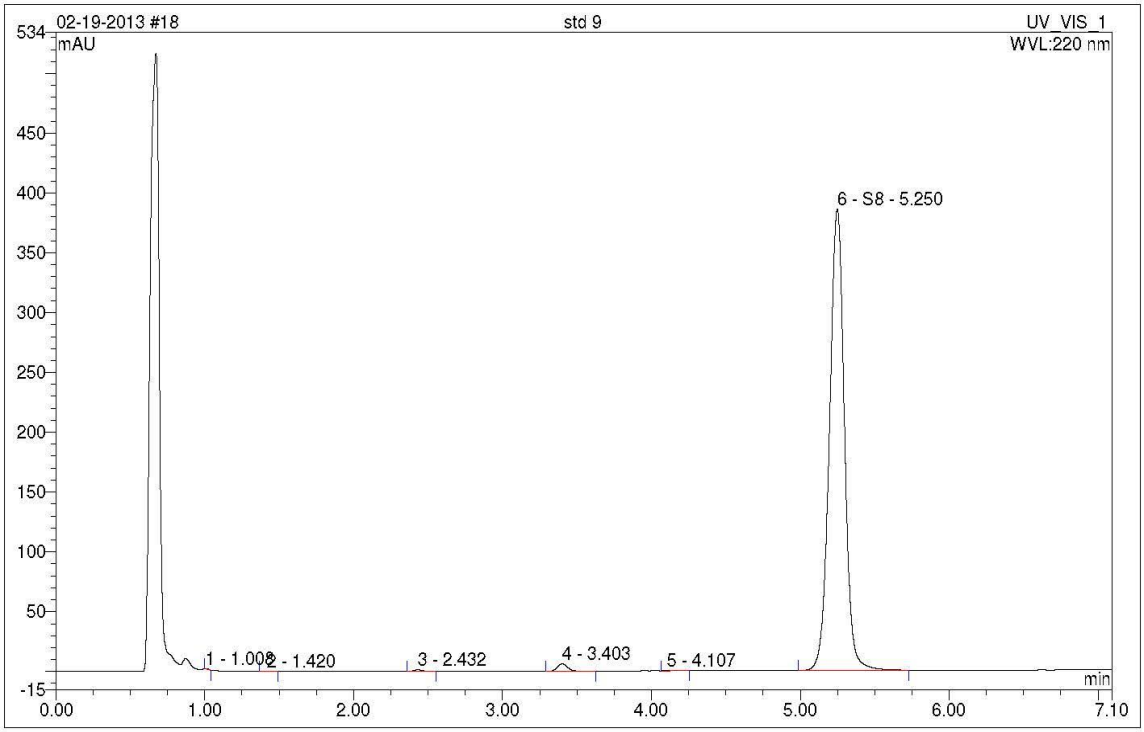
secondary standards employed in this study, the maximum area of these species combined was <2% of the S8 peak (Table 1).

The first peak at the dead volume (DV) time is injection solvent. The next peak (0.87 min) is likely a contaminant coming from the solvent as the peak areas are constant regardless of standard concentration. If it were an impurity from the S8, it would be a constant proportion of the S8 peak.

The rest of the peaks from 1.0 min to 4.1 min. are likely impurities or sulphur allotropes, as their areas are a constant proportion of the S8 peak. We expect to see S6 and S7 based on literature. These allotropes may be the peaks at 2.4 min and 3.4 min. In any case, all peaks relative to S8, when taken together, represent less than 2% of the S8.

The literature[1] indicates that there is re-arrangement in solvent from S8 to S6 and S7; therefore, 2% impurity is probably the best we can achieve. A 2% impurity will not significantly affect accuracy.

Figure 1a. Chromatogram of 200 µg/mL injection, C18 column (high capacity) – Full Scale



[1] Tebbe, F.N. *et al*., *J. Am. Chem. Soc*. 1982, 104, 4971-4972

Figure 1b. Chromatogram of 200 µg/mL injection, C18 column (high capacity) - Expanded Y-axis

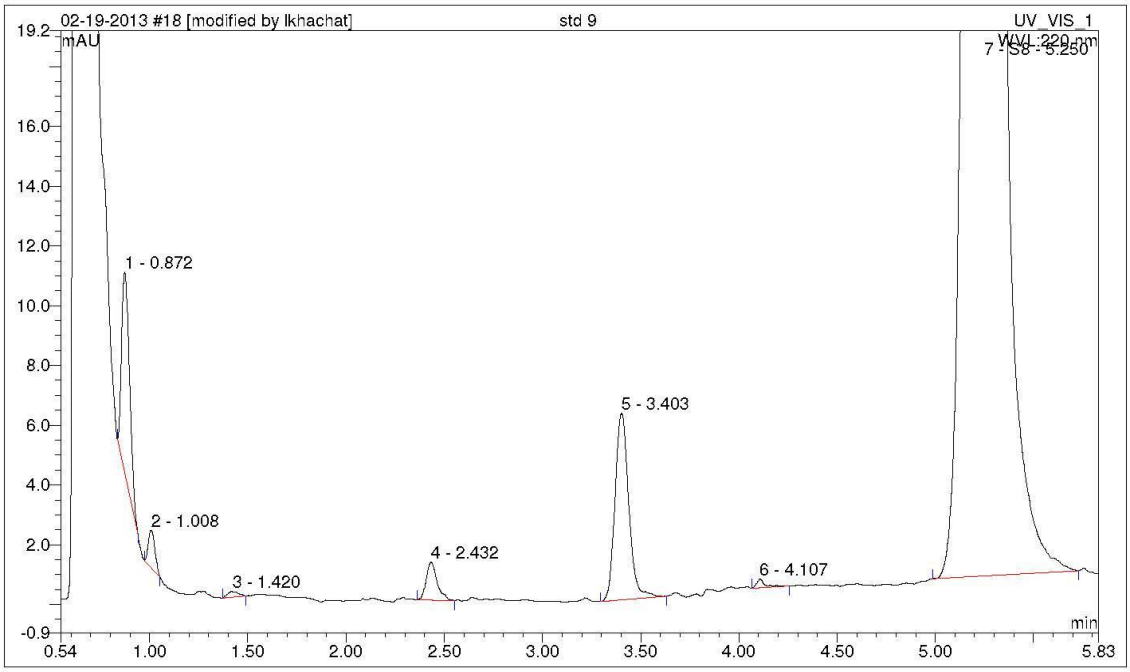


Table 1. Summary of Impurities in Sigma-Aldrich S8 Standard

Summary of Peaks and Peak Areas for Standard S8 (10 µL injected)													
id	µg/ml	0.87 min	% of S8	1.0 min	% of S8	1.4 min	% of S8	2.4 min	% of S8	3.4 min	% of S8	4.1 min	5.2 min (S8)
std 2	0.4	0.58	743.6%										0.08
std 3	2	0.54	154.3%										0.35
std 4	4	0.35	47.3%										0.74
std 5	10	0.49	19.9%										2.46
std 6	20	0.21	5.1%										4.10
std 7	40	0.3	3.4%	0.02	0.23%			0.02	0.23%	0.075	0.86%		8.70
std 8	100	0.23	1.0%	0.038	0.17%			0.038	0.17%	0.22	0.99%		22.19
std 9	200	0.32	0.7%	0.083	0.18%	0.013	0.03%	0.083	0.18%	0.51	1.14%	0.015	44.88

2.2 Evaluation of Extraction Solvents

2.2.1 Solubility

The first step was to determine the solubility of S8 in the various solvents. Three replicates were prepared in each of acetone, dichloromethane (DCM) and methanol (MeOH); one replicate was prepared in chloroform ($CHCl_3$) (Table 2). Excess S8 was mixed with solvent, sonicated for one hour, centrifuged and decanted. The supernatant was analyzed colourimetrically.

Table 2. Summary of Elemental Sulphur Solubility in Acetone, DCM, Chloroform, Methanol

Solvent	Solubility (µg/mL)	Comments
Acetone	604, 623, 610	Incomplete dissolution, very small amount of dusty sediment
Dichloromethane	6185, 6089, 6720	Incomplete dissolution, visible amount of sediment
Chloroform	6670	Incomplete dissolution, visible amount of sediment
Methanol	260, 247, 265	Incomplete dissolution, visible amount of dusty sediment

Acetone, DCM and chloroform are extraction solvents; methanol is used as the mobile phase. As can be seen, the solubility of S8 in chloroform and DCM are the same at approximately 6,500 µg/mL. Solubility in acetone is about an order of magnitude less at 600 µg/mL.

2.2.2 Extraction Efficiency

Extraction efficiency was evaluated by preparing high concentration spikes of a clay matrix (40% clay by hydrometer) containing 9% and 2% total organic carbon, respectively.

All samples were dried at 55 ± 5°C to eliminate moisture. This minimizes microbial influence on sample integrity and also reduces problems when extracting with hydrophobic solvents. The samples were then ground to < 2 mm to provide sample homogeneity.

Individual 2 g aliquots were spiked with a concentrated S8 standard in DCM. Spiked samples were mixed and the DCM allowed to evaporate overnight in the fumehood. Extraction solvent, 20 mL, was added and the samples sonicated for 30 minutes, followed by tumbling in a rotary mixer for one hour. Samples were extracted at 5:1 and 10:1 solvent:soil (volume:weight) ratios. Extracts were centrifuged, decanted and analyzed both colourimetrically and by HPLC. Recoveries using both analytical techniques were similar. Graphs of the results are displayed in Figures 2a and 2b, below.

Figure 2a. Acetone Extraction Efficiency

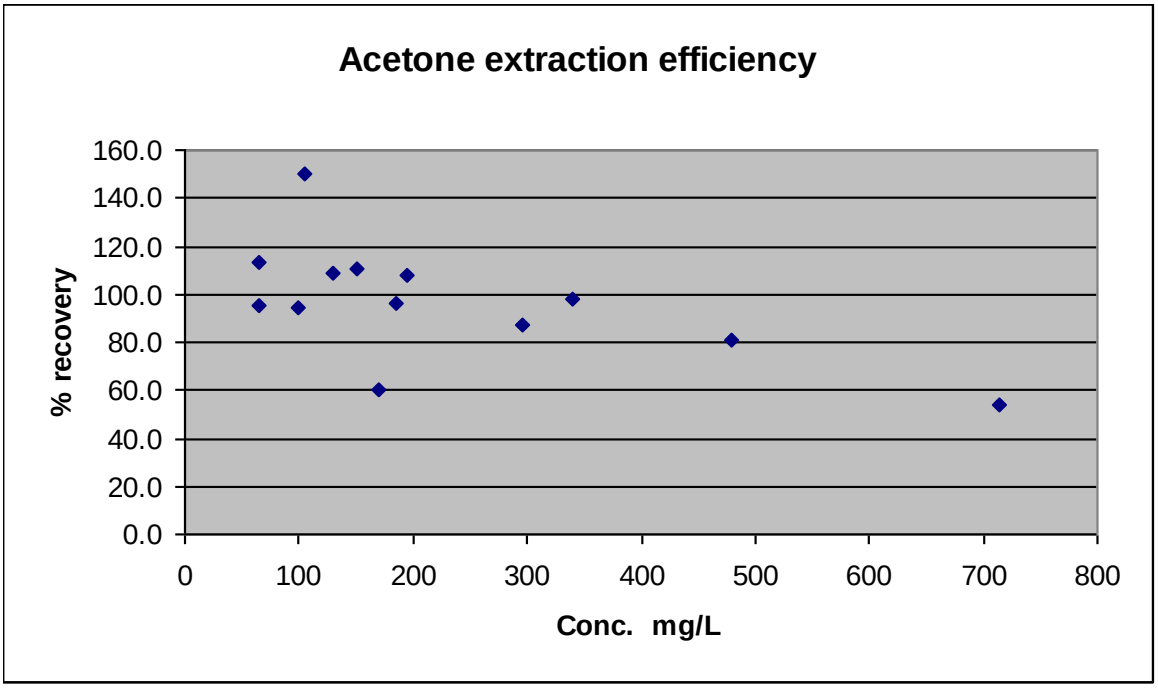
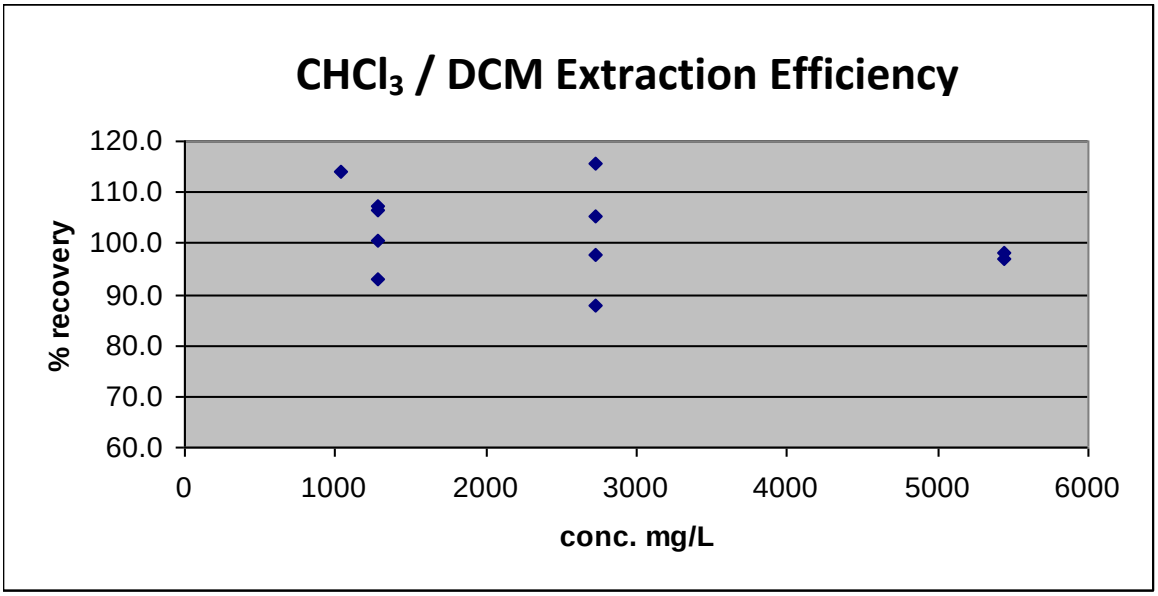


Figure 2b. Chloroform/Dichloromethane Extraction Efficiency



The data show that extraction efficiency is dependent on the solubility of S8 in the various solvents. Again, data for chloroform and DCM were similar showing 100% efficiency at spike extract concentrations up to 6,000 mg/L (extract concentration). Acetone shows excellent recoveries up to at least 400 mg/L. Going forward, depending on the solvent, if analysis yields values at these levels or above, re-extraction using a larger solvent:soil ratio would be required.

2.3 Evaluation of Instrument Response, Columns and Mobile Phases

Two systems were evaluated: 1) the Watkinson *et al*., 1987 reference method, which employs a polymeric reversed phase (PRP) column and adsorption (solid/liquid) chromatography with a chloroform-methanol mobile phase, and 2) the most commonly used analytical system, a C18 column with partition chromatography using methanol-water as the mobile phase (Azarova *et al.*, 2001).

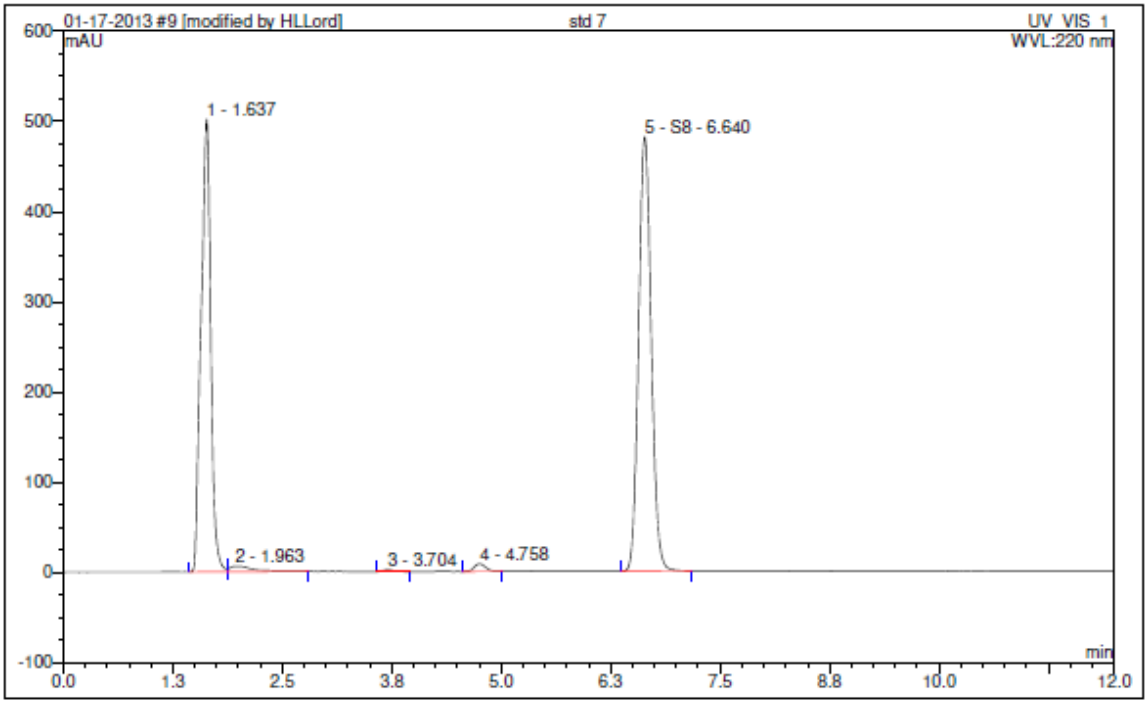
From scans of standards, it was determined that the highest molar absorptivity achievable for S8 occurs at 220 nm. Because the UV cutoff for chloroform is 240 nm, it was necessary to use the less sensitive 254 nm peak for the PRP work. The chromatograms below (Figures 3a to 3c) show the relative response of the same standard at various wavelengths using the C18 column.

Figure 3a. S8 Response at 220 nm

5 std 7

Mobile phase(MeOH:W-90:10), flow=0.5ml/min, Pressure=136bar, t=45C, column 3x150mr

Sample Name:	**std 7**	*Injection Volume:*	**10.0**
Vial Number:	**BB7**	*Channel:*	**UV_VIS_1**
Sample Type:	**standard**	*Wavelength:*	**220**
Control Program:	**S8-11**	*Bandwidth:*	**1**
Quantif. Method:	**S8**	*Dilution Factor:*	**1.0000**
Recording Time:	**1/17/2013 17:31**	*Sample Weight:*	**1.0000**
Run Time (min):	**12.00**	*Sample Amount:*	**1.0000**



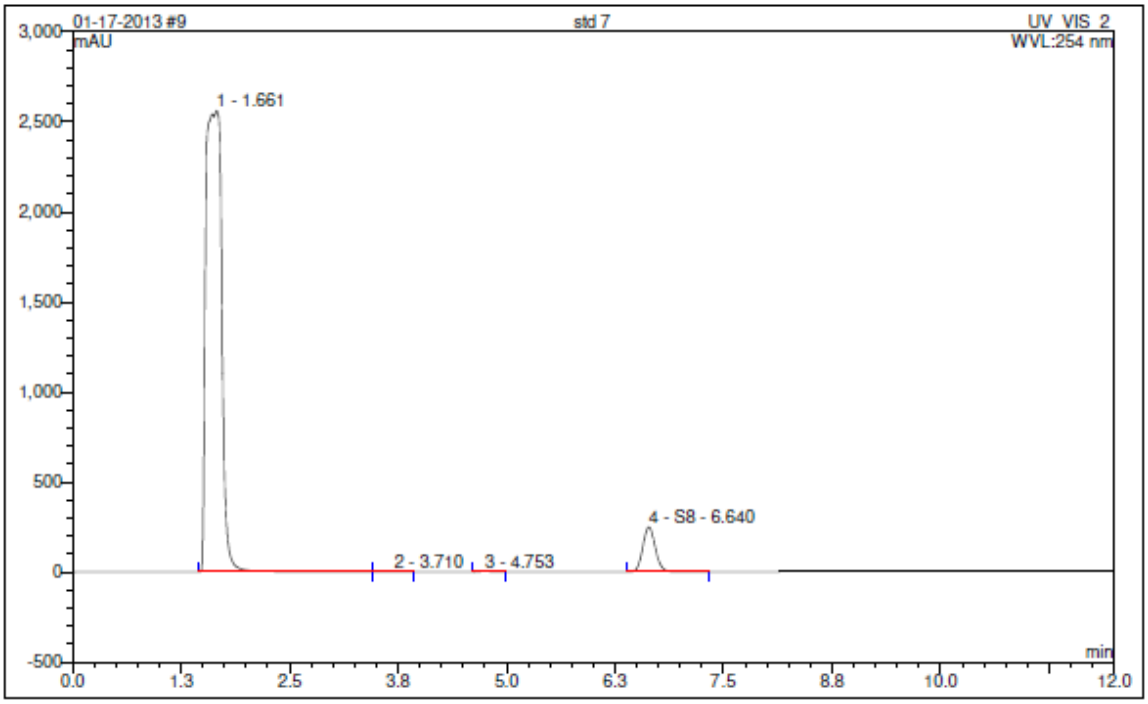
No. V_VIS_	Ret.Time UV_VIS_1 min	Peak Name UV_VIS_1	Height UV_VIS_1 mAU	Area UV_VIS_1 mAU*min	Rel.Area UV_VIS_1 %	Amount UV_VIS_1 ug/ml	Type UV_VIS_1
1	1.64	n.a.	501.840	64.560	43.40	n.a.	BM
2	1.96	n.a.	6.127	1.978	1.33	n.a.	MB
3	3.70	n.a.	2.112	0.293	0.20	n.a.	BMB
4	4.76	n.a.	8.719	1.327	0.89	n.a.	BMB
5	6.64	S8	481.698	80.602	54.18	100.399	BMB
Total:			1000.496	148.761	100.00	100.399	

Figure 3b. S8 Response at 254 nm

4 std 7

Mobile phase(MeOH:W-90:10), flow=0.5ml/min, Pressure=136bar, t=45C, column 3x150mm

Sample Name:	**std 7**	*Injection Volume:*	**10.0**
Vial Number:	**BB7**	*Channel:*	**UV_VIS_2**
Sample Type:	**standard**	*Wavelength:*	**254**
Control Program:	**S8-11**	*Bandwidth:*	**1**
Quantif. Method:	**S8**	*Dilution Factor:*	**1.0000**
Recording Time:	**1/17/2013 17:31**	*Sample Weight:*	**1.0000**
Run Time (min):	**12.00**	*Sample Amount:*	**1.0000**



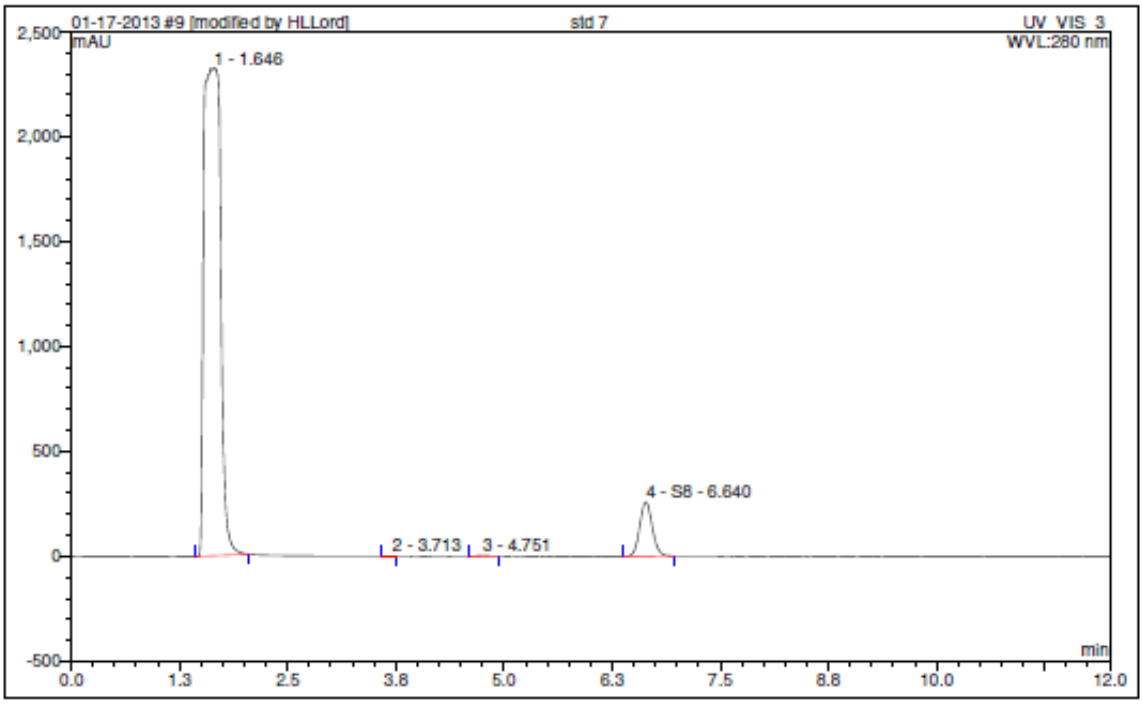
No. V_VIS_	Ret.Time UV_VIS_2 min	Peak Name UV_VIS_2	Height UV_VIS_2 mAU	Area UV_VIS_2 mAU*min	Rel.Area UV_VIS_2 %	Amount UV_VIS_1 ug/ml	Type UV_VIS_2
1	1.66	n.a.	2561.394	540.600	92.71	n.a.	BM
2	3.71	n.a.	1.298	0.202	0.03	n.a.	MB
3	4.75	n.a.	3.948	0.585	0.10	n.a.	BMB
4	6.64	S8	248.725	41.738	7.16	100.399	BMB
Total:			2815.365	583.125	100.00	100.399	

Figure 3c. S8 Response at 280 nm

4 std 7

Mobile phase(MeOH:W-90:10), flow=0.5ml/min, Pressure=136bar, t=45C, column 3x150mm

Sample Name:	**std 7**	*Injection Volume:*	**10.0**
Vial Number:	**BB7**	*Channel:*	**UV_VIS_3**
Sample Type:	**standard**	*Wavelength:*	**280**
Control Program:	**S8-11**	*Bandwidth:*	**1**
Quantif. Method:	**S8**	*Dilution Factor:*	**1.0000**
Recording Time:	**1/17/2013 17:31**	*Sample Weight:*	**1.0000**
Run Time (min):	**12.00**	*Sample Amount:*	**1.0000**



No. V_VIS	Ret.Time UV_VIS_3 min	Peak Name UV_VIS_3	Height UV_VIS_3 mAU	Area UV_VIS_3 mAU*min	Rel.Area UV_VIS_3 %	Amount UV_VIS_1 ug/ml	Type UV_VIS_3
1	1.65	n.a.	2324.968	517.138	92.21	n.a.	BMB*
2	3.71	n.a.	0.354	0.030	0.01	n.a.	BMB*
3	4.75	n.a.	3.043	0.414	0.07	n.a.	BMB
4	6.64	S8	259.007	43.239	7.71	100.399	BMB
Total:			2587.372	560.822	100.00	100.399	

Analysis using a chloroform mobile phase and polymeric column was plagued with difficulty. The column is a specialty item and took several weeks to obtain. The chloroform attacked the pump seals leaving the system not functional until the Teflon® seals were obtained and installed (two weeks). Limited data showed adequate separation of the S8 peaks and adequate sensitivity.

However, since the mandate was to develop a method that could be easily adopted by other commercial labs and, more importantly, conventional partition chromatography using a standard C18 column showed equal or better performance, efforts focused on the development of that method.

Example chromatograms (Figures 3d to 3h) from the two methods show the improved chromatography using C18 methanol-water separation relative to the PRP chloroform-methanol system.

Figure 3d. C18 Method – High Organic Clay in Acetone (2 µg/mL)

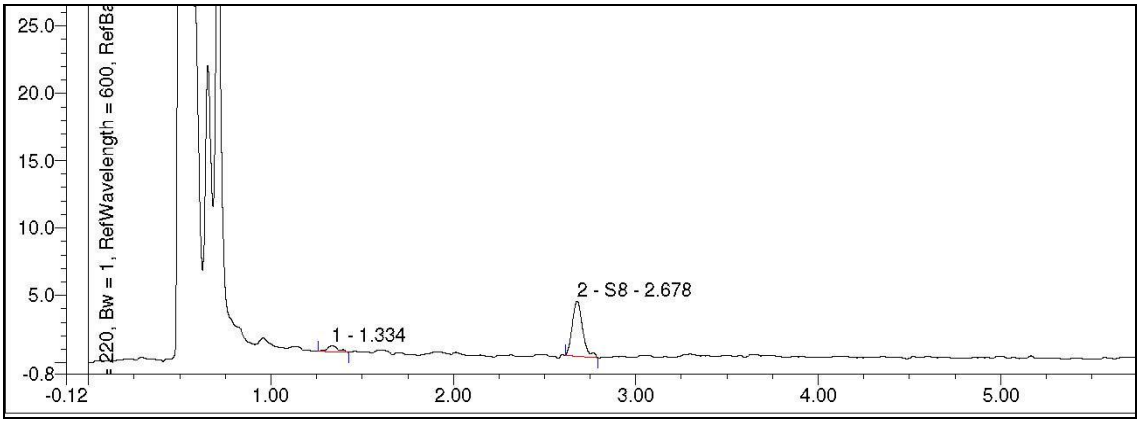


Figure 3e. PRP Method – High Organic Clay in Acetone (2 µg/mL)

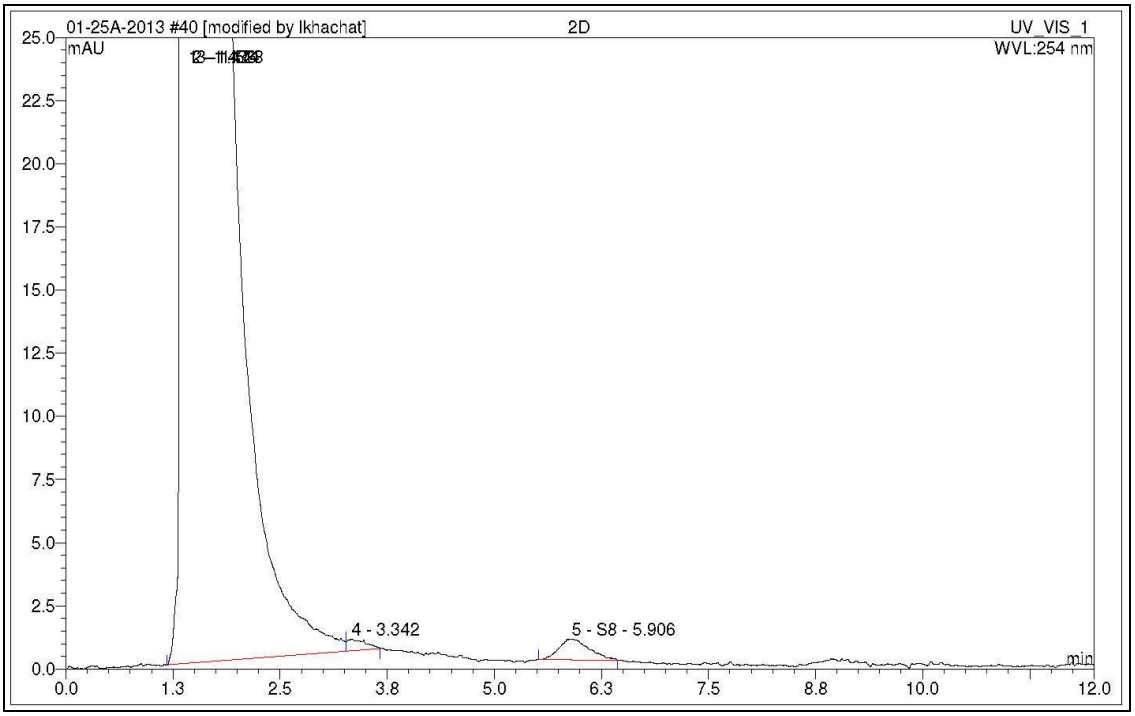


Figure 3f. PRP Method – Lodgepole Pine Litter in Acetone (10 g/mL)

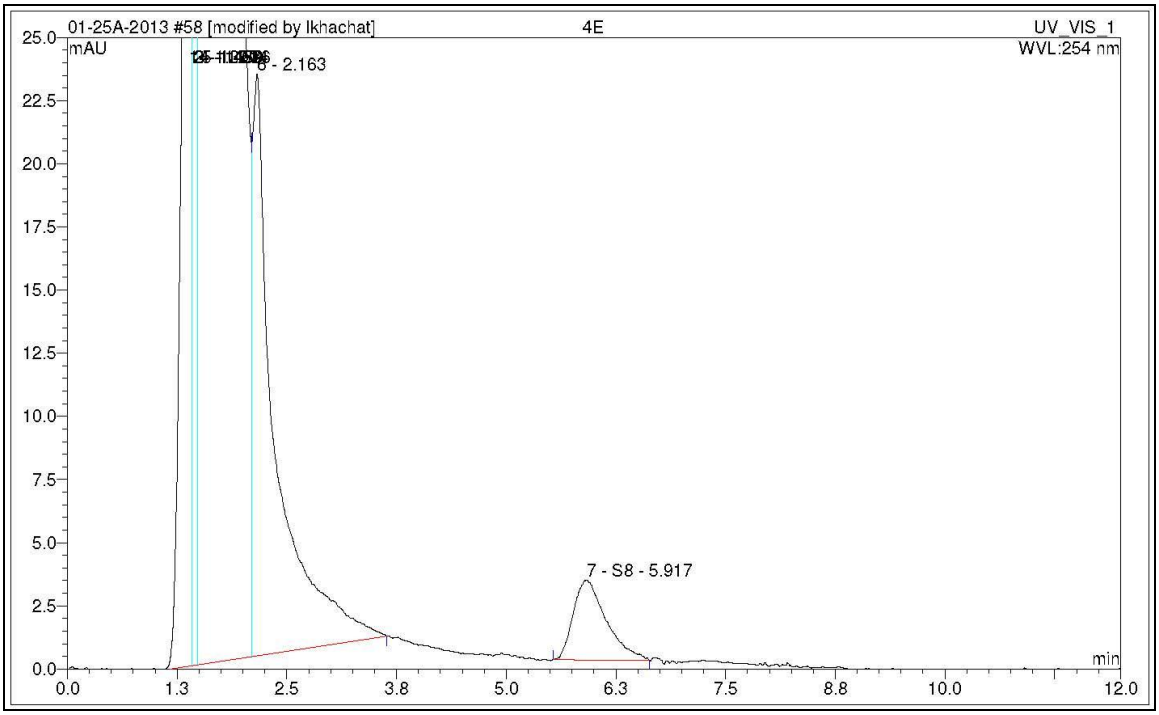


Figure 3g. C18 Method – Lodgepole Pine Litter in Acetone (10 µg/mL)

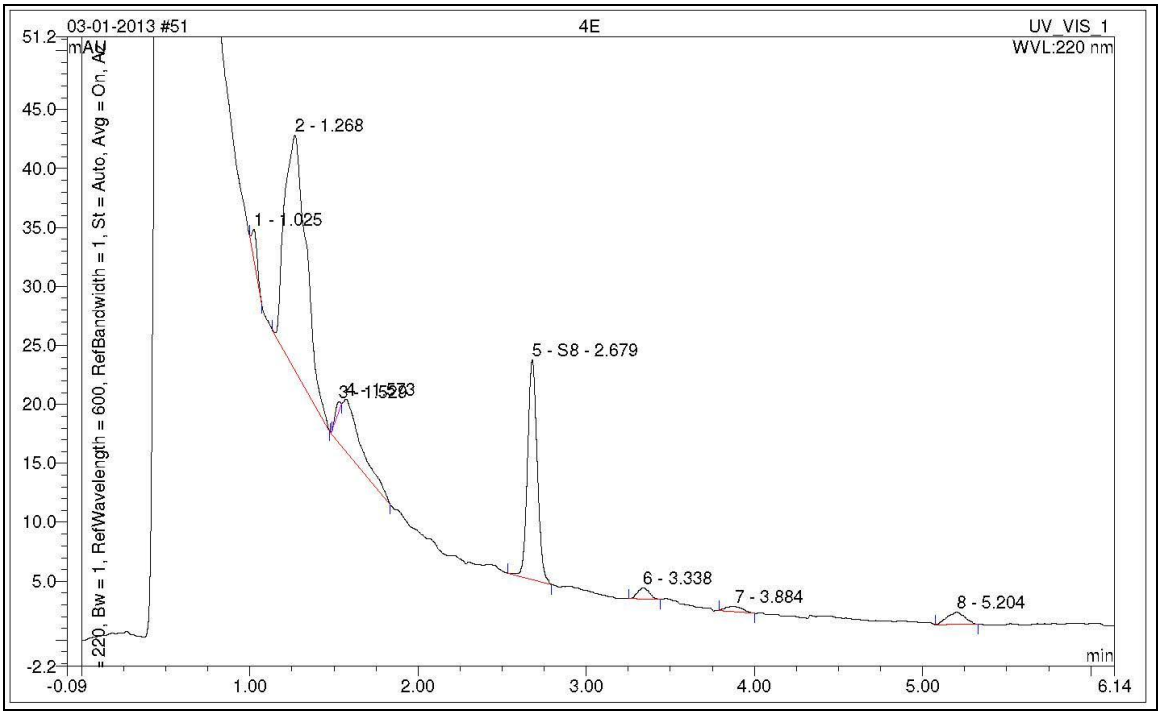
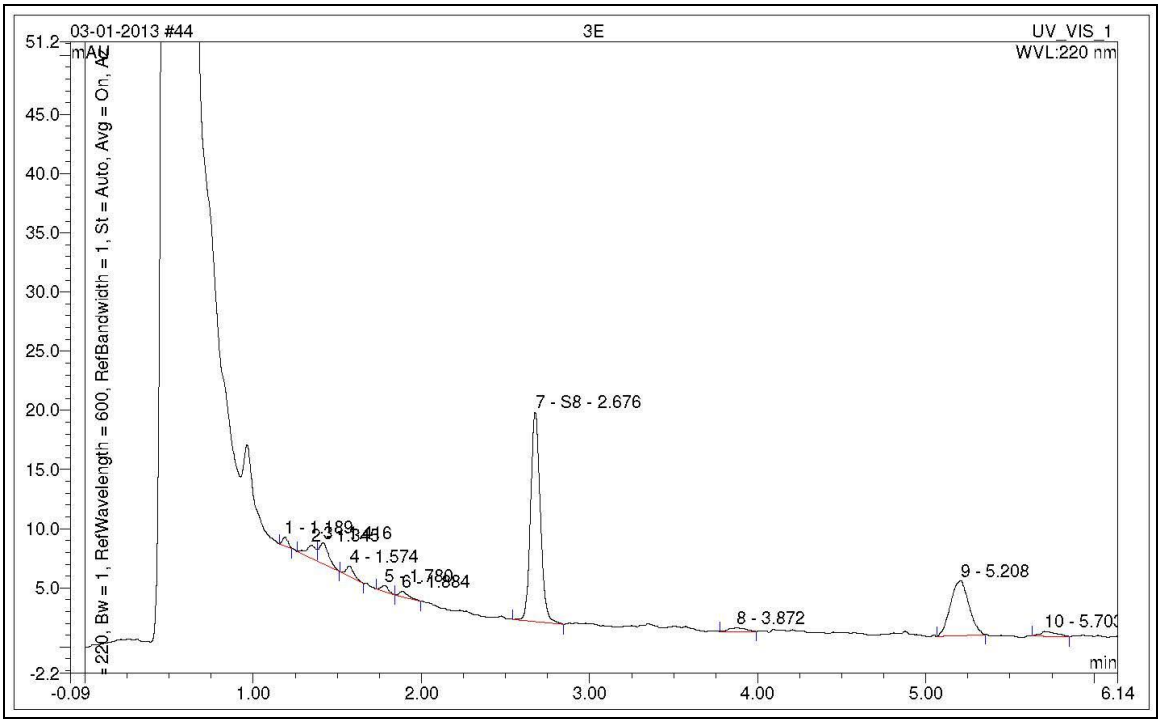


Figure 3h. C18 Method – White Spruce Litter in Acetone (10 µg/mL)



2.4 Instrument Detection Limits

Instrument detection limits (IDL) were estimated by replicate injections of a low level standard or a 3:1 signal to noise ratio (10 µL injected).

- IDL PRP / $CHCl_3$, 0.4 µg/mL
- IDL C18 / 90:10 MeOH:H_2O, 0.08 µg/mL

Note that these are IDLs and actual MDLs determined when replicates are carried through the entire analytical process will be higher. Nonetheless, the five-fold improvement in sensitivity using the C18 column and detection at 220 nm was very encouraging.

2.5 Initial Instrument Conditions

For method optimization, we targeted isocratic elution conditions that provided a capacity factor (retention factor, *k'*) about 5.[2] This was selected after reviewing the capacity factors employed by Watkinson *et al*., for soil extraction (*k'* = 5.2), and Azarova *et al*., for sediment extraction (*k'* = 5.4).

For the PRP method, a Hamilton PRP-1 column was selected (4.6 x 15 mm, 5 µm particle size). The 4.1 x 150 mm, 10 µm particle size column specified by Watkinson *et al.* was not available.

[2] Capacity factor: $k' = (t_R - t_M)/t_M$

Isocratic elution with 1:1 $CHCl_3$:MeOH (1.0 mL/min) yielded *k'* = 2.9 and retention time (t_R)= 5.9 min. Given that the S8 peak was asymmetrical and very broad (likely due to the solid/liquid interaction energetics) and the S8 peak was baseline-separated from the interference in the worst-case leaf-litter sample, conditions providing a larger *k'* were not investigated.

For the C18 method, initially a Waters X-Bridge C18 column was employed (3.0 x 150 mm, 3.5 µm particle size). Isocratic elution conditions with 90:10 MeOH:H_2O at 0.5 mL/min provided *k'* = 2.9 and t_R = 6.2 min with excellent peak shape. For enhanced capacity and resolution (and reduced column cost) an AkzoNobel Kromasil® C18 column (4.6 x 100 mm, 3.5 µm particle size) was employed later. Isocratic elution with 90:10 MeOH:H_2O at 2.3 mL/min provided *k'* = 7.7 and t_R = 5.2 min. Again peak shape was acceptable, but such high values of *k'* are typically not preferred due to their negative impact on peak resolution. Increasing eluent strength to 95:5 MeOH:H_2O while maintaining flow of 2.3 mL/min provided *k'* = 4.4 and t_R = 2.7 min. Under these conditions, there was some co-elution with the tail of polar interferences from pine and spruce leaf litters. Further refinements in elution strength will be undertaken to optimize retention and separation from co-extracted interferences in the final method validation. It may be necessary to return to a 15 cm column if the desire is to have baseline separation between interferences and S8 in all cases.

2.5.1 Initial Conclusions

- For S8 dissolved in solvent, detection at 220 nm with methanol-water mobile phase provides the best sensitivity. (Note*:* UV cutoffs for methanol, acetone, DCM and chloroform are, respectively: 205, 330, 230, and 240 nm.)
- We cannot add acetone to the C18 mobile phase to increase S8 solubility because of the 330 nm UV cutoff.
- For the PRP method, we will lose about 50% of sensitivity by having to detect at the higher wavelengths.

2.6 Sensitivity and Freedom from Interferences

2.6.1 Sample Extraction

Sensitivity and freedom from interferences were evaluated by low level spikes of the high and low organic clays and three leaf litter samples provided by ESRD. The following approaches were used:

- Two clay samples and three leaf litter samples extracted with three solvents at 5:1 and 10:1 solvent:soil ratios,
- 2 g soil and 10 or 20 mL solvent used,
- Spiked at 20 and 100 µg/g S8 in the soil, corresponding to 2 to 10 µg/mL S8 in the 10:1 extract and 4 and 20 µg/mL S8 in the 5:1 extract.

All samples were extracted and centrifuged as described in Section 2.2.2. The leaf litter samples proved more challenging, particularly for the DCM and chloroform extracts. Because of the density of these solvents, significant floating and suspended materials remained after centrifuging. These samples had to be centrifuged a second time and some were filtered before analysis. Also, the 5:1 extracts were more difficult to separate than the 10:1. In contrast, the acetone extracts settled readily and could be decanted after a single centrifugation.

The leaf litter extracts were all highly coloured. The high and low organic clay samples were colourless. Example chromatograms of unspiked 10:1 acetone extracts using an expanded y-axis scale show little interference in the S8 elution range, indicated by the red marker (Figures 4a to 4e). Since acetone extracts were run undiluted, these represent the worst-case scenario. As expected, the leaf litter samples displayed significant interferences, but these mostly elute prior to S8. Therefore, the mobile phase is capable of separating the signals associated with the interference from those of S8. A chromatogram of 20 µg/mL S8 standard in acetone is presented for comparison (Figure 4f).

Figure 4a. High Organic Clay

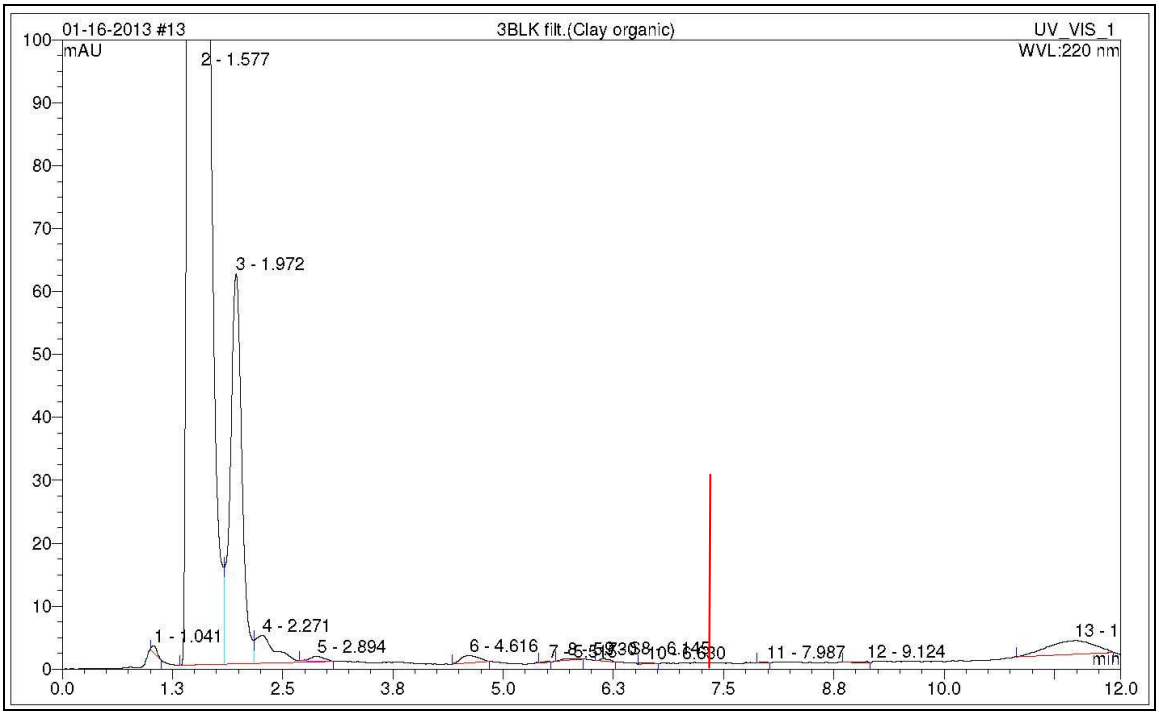


Figure 4b. Clay

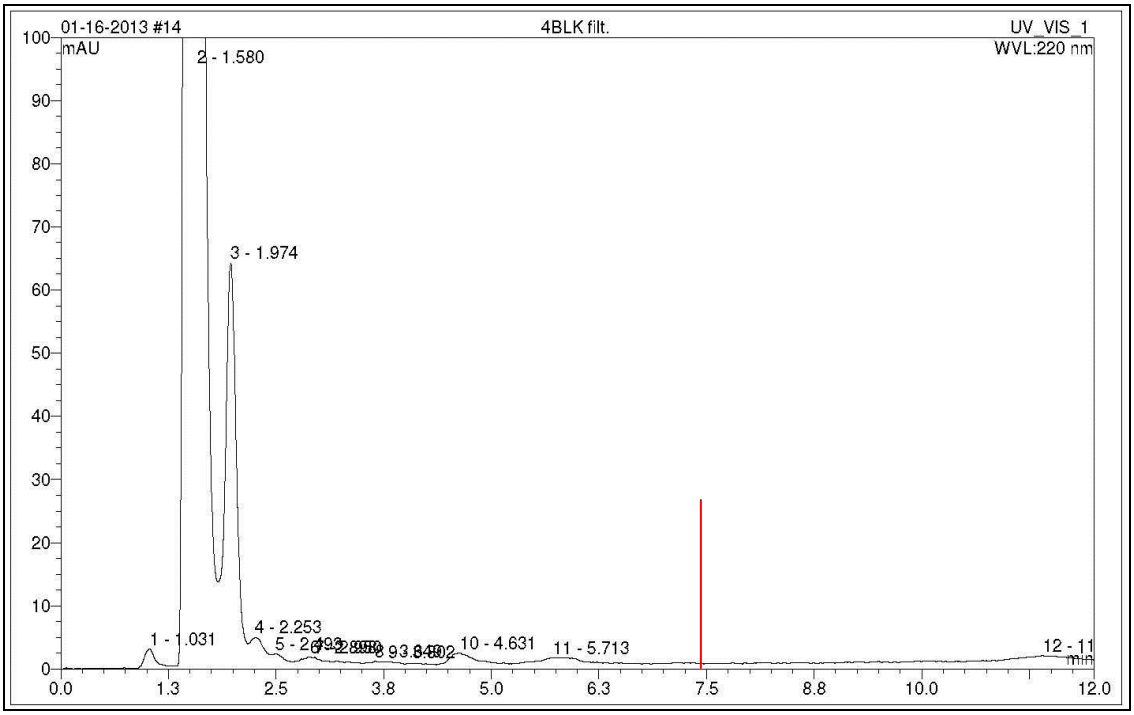


Figure 4c. White Spruce Leaf Litter

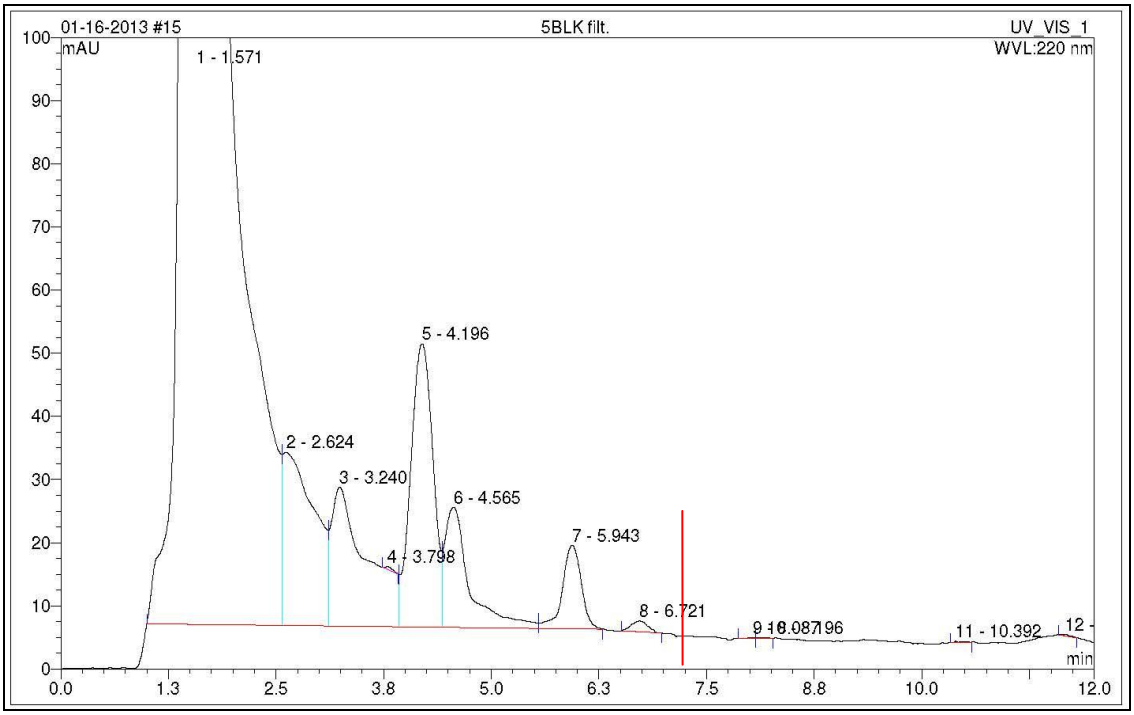


Figure 4d. Lodgepole Pine Leaf Litter

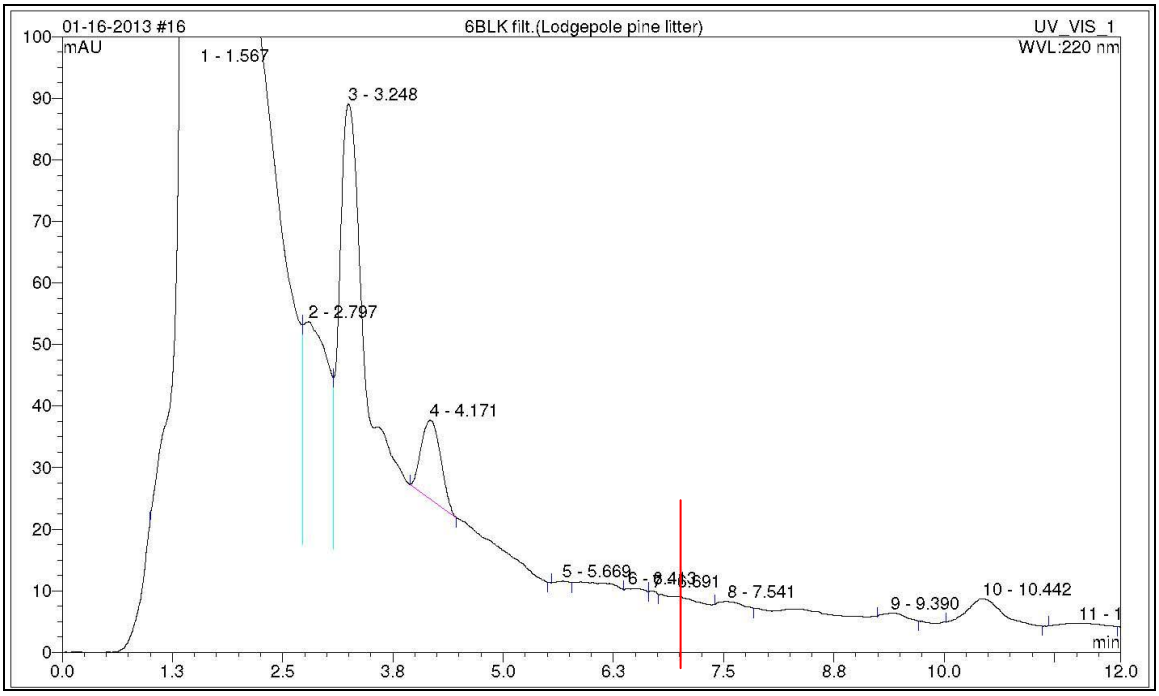


Figure 4e. Aspen Leaf Litter

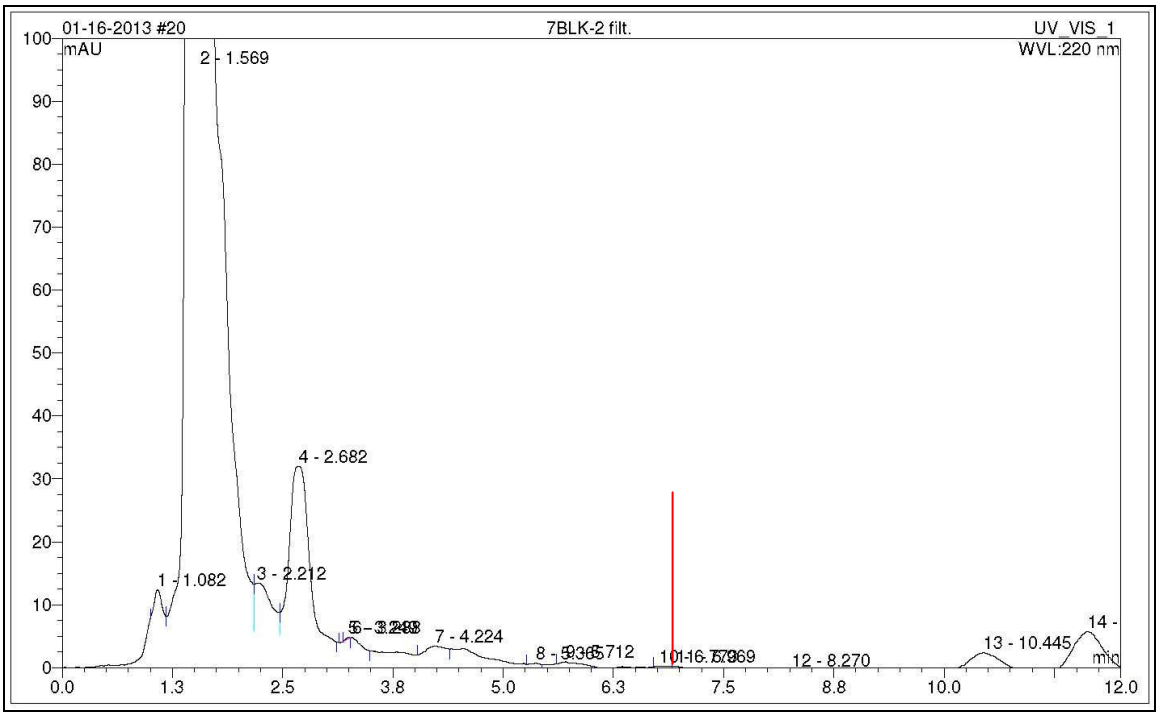
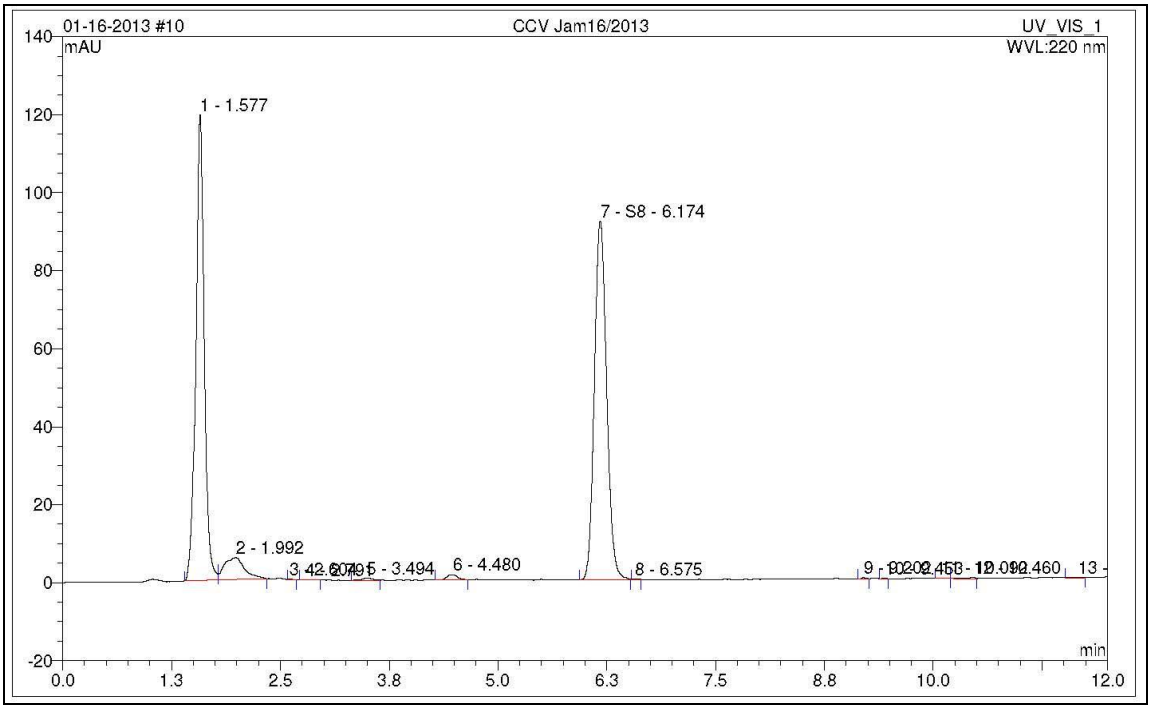


Figure 4f. S8 Standard in Acetone (20 µg/mL)



All three solvent extracts were analyzed by HPLC. Acetone extracts were also analyzed colourimetrically. The data are tabulated in the Master Data Table in Appendix 1.

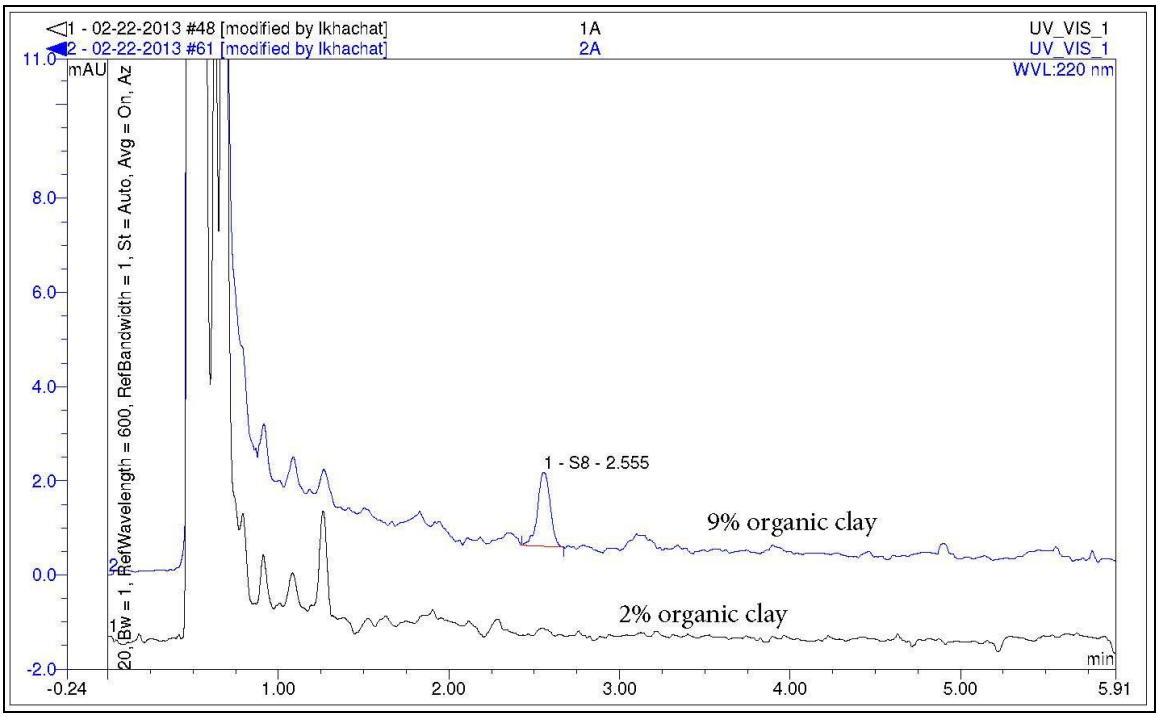
2.6.2 Colourimetric Analysis and Interference

The colourimetric analyses showed good recoveries for the clay samples, averaging 107 ± 14 %. Note that the unspiked clay samples gave small positive readings (0.5 µg/mL) by the colourimetric method. HPLC analysis confirmed a similar level (0.9 µg/mL) of S8 in the high organic clay sample (9% organic clay) but not in the low organic clay sample (2% organic clay) (see Figure 5). Taking this into account, the average recovery was 97%.

As expected, colourimetric analysis of the leaf litter extracts showed significant background levels, ranging from 2.4 to 7.7 µg/mL. These levels were not confirmed by HPLC, thus this is a true background interference, resulting in high and erratic recoveries in the spikes.

Attempts were made to correct the data by background subtraction, but this did not improve the recoveries. Thus, colourimetric analysis is not suitable for coloured extracts.

Figure 5. Sulphur Recovery in Unspiked High Organic (9%) and Low Organic (2%) Clay Samples



2.6.3 HPLC Parameter Adjustments

A 90:10 MeOH:H_2O mobile phase with a 15 cm C18 column provided clear separation of the S8 peak from all interferences while maintaining a reasonably short run time of < 12 minutes (Figures 6a to 6c).

Figure 6a. White Spruce Leaf Litter Blank Soil, Extract Spiked

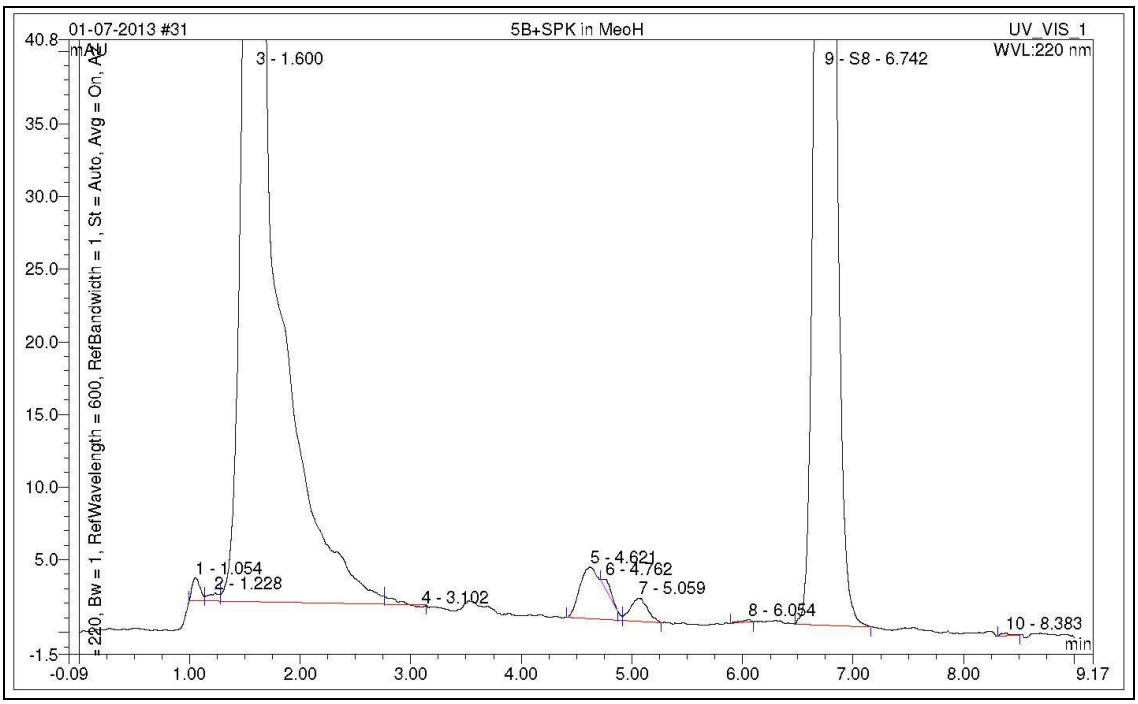


Figure 6b. Lodgepole Pine Leaf Litter Blank Soil, Extract Spiked

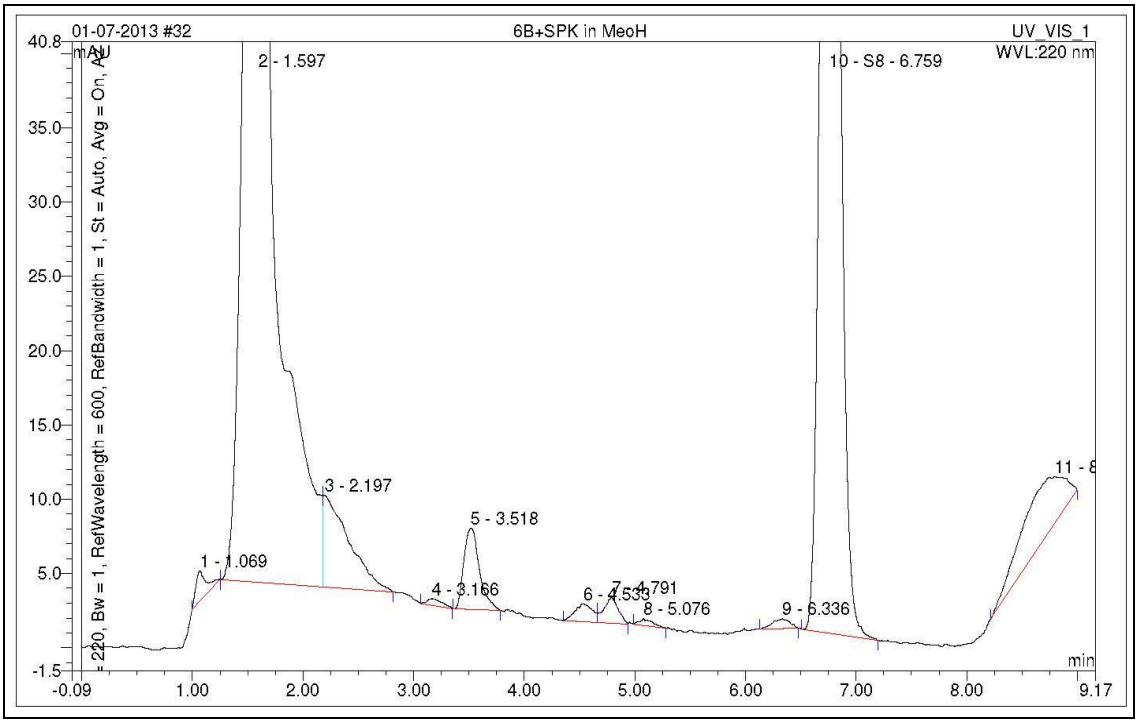
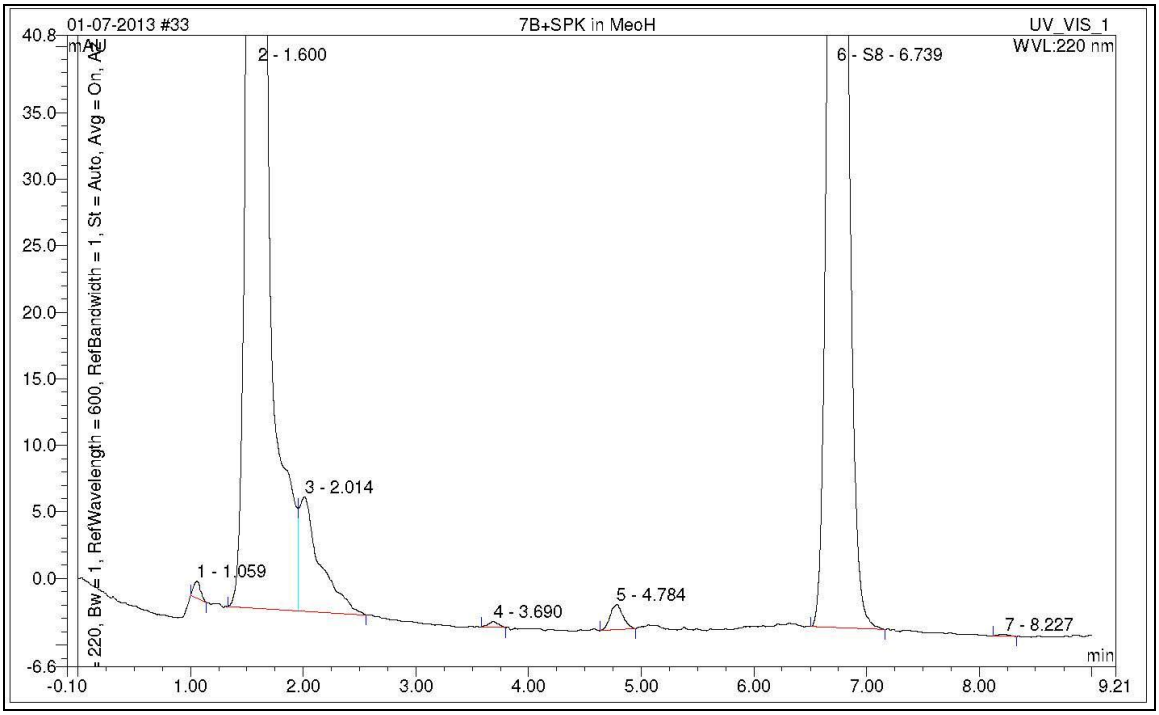


Figure 6c. Aspen Leaf Litter Blank Soil, Extract Spiked



The linear range will be limited by the solubility of S8 in the mobile phase. In the initial investigations, linearity has been established up to 200 µg/mL using the standard 10 µL extract injection. The upper limit of the linear range will be established during validation.

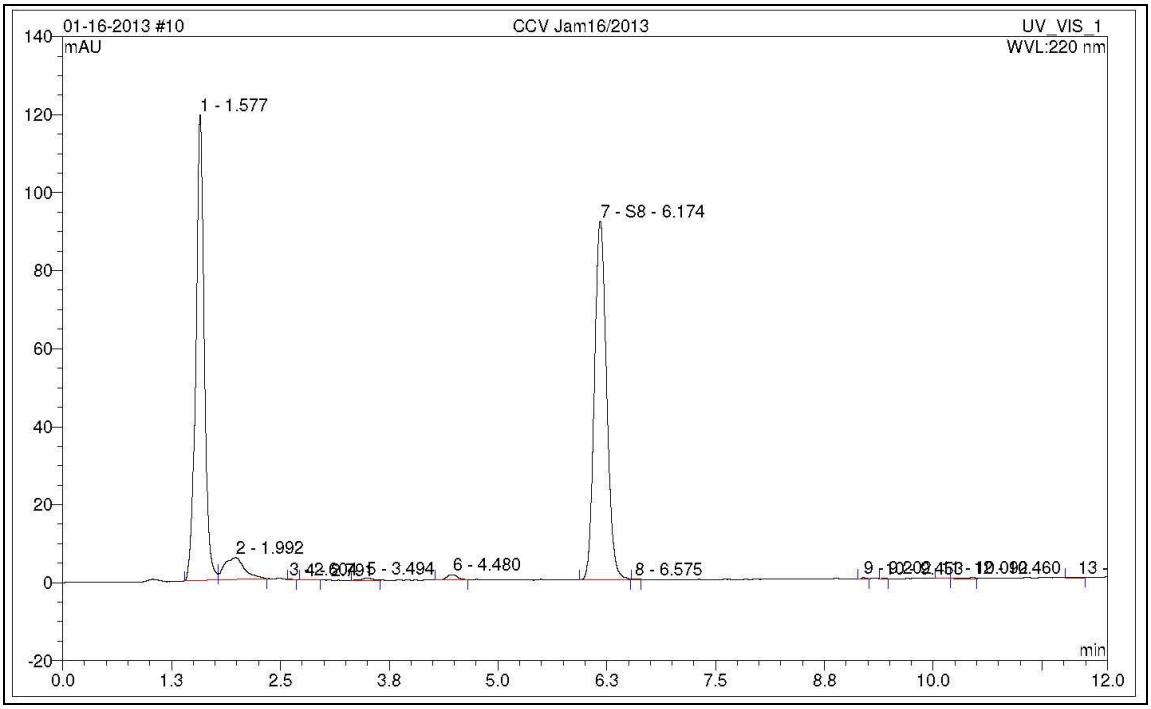
Using these protocols, and assuming a 10:1 solvent:soil ratio, the working range for the acetone extracts is estimated to be 2 – 3,000 µg/g S8 and 20 to 30,000 µg/g (3%) S8 for DCM extracts.[3] This assumes linearity can be extended to 300 µg/mL in the extracts. Early indications are that linearity may extend to 500 µg/mL, which is near the solubility limit of S8 in acetone.

2.6.4 Sample Dilution and Analysis

Acetone extracts were run undiluted. DCM and chloroform extracts were diluted 10x with acetone prior to injection to ensure miscibility of the chlorinated solvents in the mobile phase. Note that the 9 or 10 µL of acetone injected is insufficient to interfere with the S8 peak. In the example chromatogram below (Figure 7) the large peak at 1.6 min. is due to acetone.

[3] DCM (and chloroform) extracts are diluted 10x in either methanol or acetone prior to injection. Neat DCM and chloroform are not miscible with the methanol-water mobile phase. We observed no miscibility problems during chromatography with the chlorinated solvent extracts when diluted as described. Dilution in acetone provides a means to accommodate the solvent with higher S8 solubility and so is preferred for higher concentration extracts. We recommend acetone as diluent going forward.

Figure 7. S8 Standard in Acetone (20 µg/mL)



All three extraction methods provided good recoveries. Table 3 gives the averages and standard deviations for the recoveries of the five samples using three different solvents.

Table 3. Average Sulphur Recovery - All Solutions (DCM, Acetone, Chloroform)

Extract Ratio (Solvent to Soil)	Design µg/mL	Average Recovery	Standard Deviation
10 to1	2	130% (106%)	26%
5 to 1	4	104%	23%
10 to 1	10	88%	11%
5 to 1	20	87%	12%

The chloroform extracts exhibited a high bias on the 2 µg/mL extracts, which may be an artifact since these were analyzed some time after the rest. Excluding these values, the mean recovery was 106%.

The larger variability observed for the most dilute extracts, 2 µg/mL and 4 µg/mL, is in keeping with samples having concentrations near the detection limit. A chromatogram of standards at 0.4 µg/mL (std 1), 1.0 µg/mL (std 2) and 2.0 µg/mL (std 3) is shown in Figure 8a. Example chromatograms of 2 µg/mL extracts are shown in Figures 8b to 8d below.

Figure 8a. Standards at 0.4, 1.0, 2.0 µg/mL

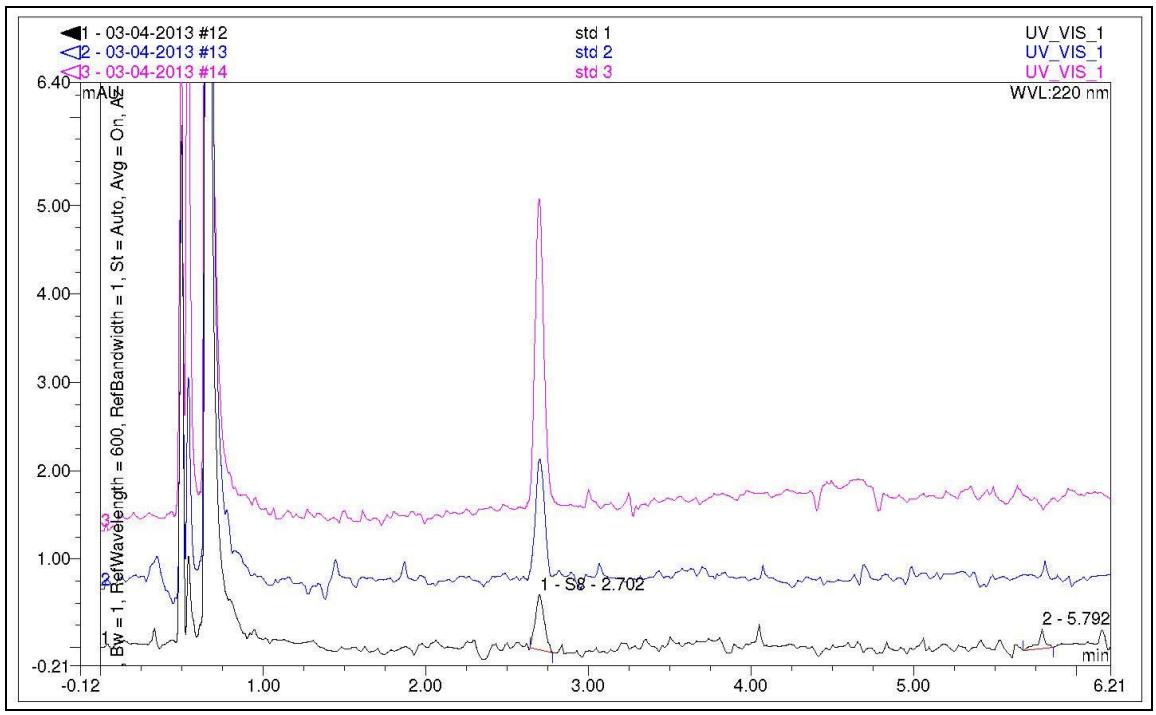


Figure 8b. High Organic Clay Spiked, Extract at 2 µg/mL

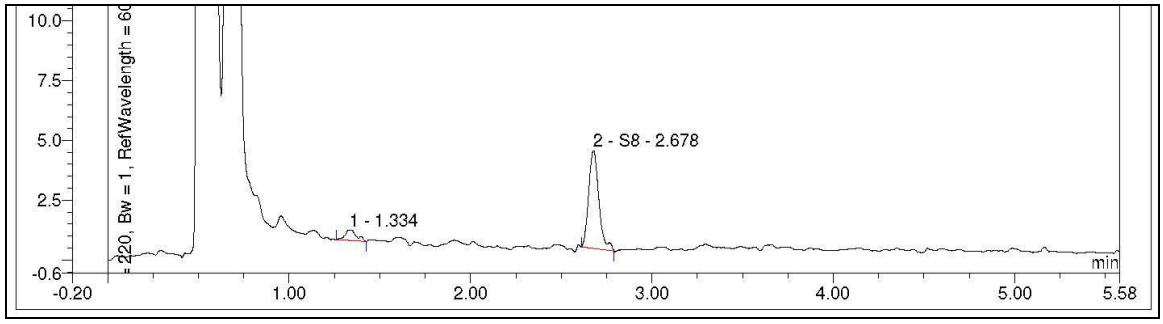


Figure 8c. Lodgepole Pine Leaf Litter Spiked, Extract at 2 µg/mL

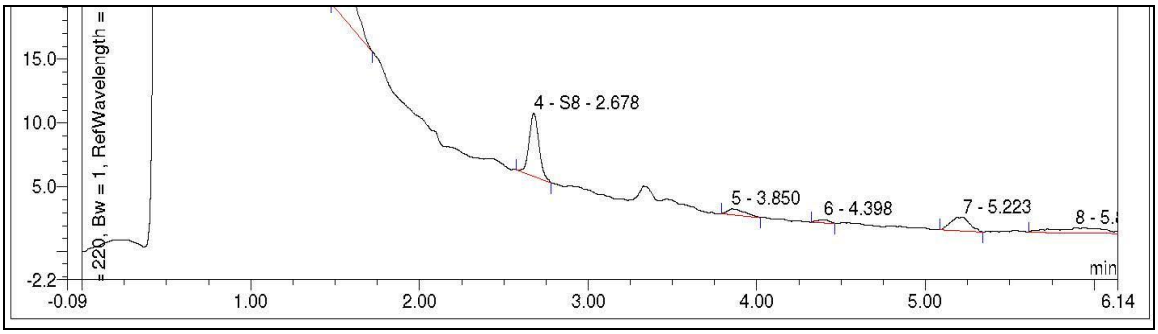
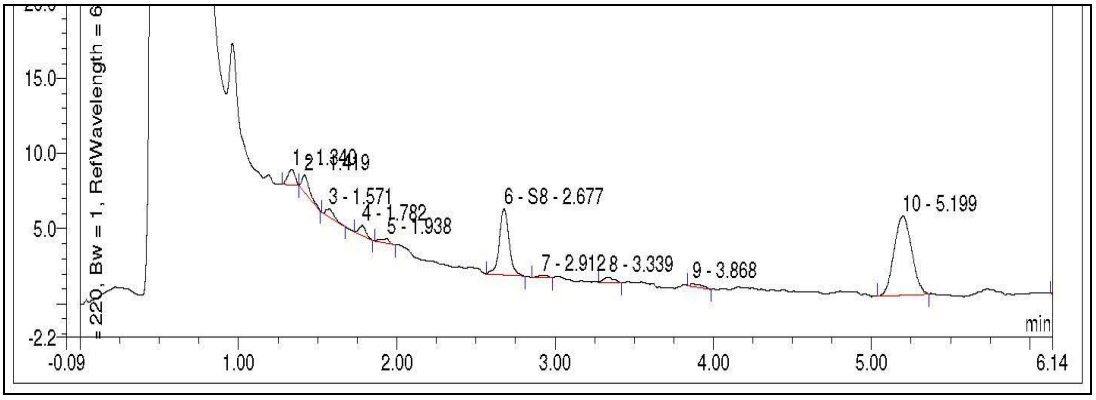


Figure 8d. White Spruce Leaf Litter Spiked, Extract at 2 µg/mL



The average recoveries for all extracts determined using each of the tested solvents (excluding the 2 µg/mL chloroform extracts) are shown in Table 4.

Table 4. Average Recoveries in Acetone, DCM and Chloroform

Solvent	Average Recovery	Number of Replicates
Acetone	98%	12
DCM	103%	12
Chloroform	101%*	8

*excluding 2 µg/mL extracts

2.6.5 Robustness

Relatively rapid clogging of the column and concomitant backpressure build-up was observed. This was probably due to pine pitch from the leaf litter extracts, not particulate matter in the extracts, as a 2 µm in-line filter was employed immediately upstream of the column and extracts were filtered prior to injection. In response, various guard columns and column cleaning regimes were investigated. The optimum guard column and cleaning protocol will be included in the final SOP. At this point it appears that the lodgepole pine extracts cause the worst column contamination. Cleaning with polar solvents (acetonitrile, acetone, methanol) either on their own or in various combinations with water is not sufficient to eliminate the contamination. Isopropanol, hexane and/or heptane may be moderately effective. Investigations were conducted to identify a guard column that is most effective in trapping the interference in order to avoid having to implement either a periodic column-cleaning regimen or gradient elution with a suitable solvent to elute the interference at the end of each run.

2.7 Selection of Protocol for Validation

The preceding data was presented to ESRD, a protocol selected for optimization, and, after optimization, the method was documented in a standard operating procedure. The reasoning for the protocol selection is detailed in Section 3 below.

3 Development of the Final Optimized Analytical Method and Recommended Operating Protocols

Based on the findings of the initial investigations and consultation with ESRD, method optimization and validation were conducted on the following methodology:

- Extraction with acetone and DCM using 2 g soil and 20 mL solvent. Analysis using reversed phase partition HPLC with a conventional C18 column, methanol-water mobile phase and UV detection at 220 nm.

The reasoning for these choices is as follows:

- Government of Alberta requires a high level method for sites heavily impacted by elemental sulphur and a low level method for remote areas less affected by longer range aerial transport. Thus, two solvents were validated for use: DCM for high levels because of the much greater solubility of S8, and acetone for low levels because the extract can be run undiluted.
- DCM was chosen over chloroform because it provides equal performance to chloroform, is a common laboratory solvent and is not a carcinogen.
- A 10:1 solvent:soil ratio was adopted as the optimum compromise between providing adequate sensitivity and effective extraction and separation of the extract from the solid matrix.
- The conventional C18 column was chosen instead of the PRP because it is widely used in the laboratory community and provides superior sensitivity to the PRP.

3.1 HPLC Method Optimization

3.1.1 Optimization of the Instrumental Response

Selection of the 220 nm wavelength was discussed in Sections 2.3 to 2.4. Optimization of other instrumental parameters is discussed below.

3.1.2 Optimization of the Mobile Phase

The most difficult sample matrix was lodgepole pine leaf litter. Although the original work provided reasonable separation of the S8 peak from interferences, additional work was done to improve the separation. Optimization included varying the methanol to water ratio to further increase the capacity factor while maintaining a reasonably short run time. The optimized

system is a compromise between separation and a realistic run time. The chromatogram at normal scale expansion shows virtual complete separation from the interference. Expanded scale shows that < 1% of the interference remains at the S8 elution time but that a peak of a very low level standard is easily established.

Selectivity Evaluation

- Mobile phase 95:5 MeOH:H_2O, *k'* = 4.4
- Mobile phase 90:10 MeOH:H_2O, *k'* = 7.4

We found the worst-case scenario for selectivity occurs for acetone extraction/injection of lodgepole pine soil samples. The acetone extracts were injected undiluted so a significantly higher amount of co-extracted interference was injected relative to the DCM extracts diluted 10x prior to injection. Of the soils tested, lodgepole pine has the most challenging interferences. These are, however, primarily polar and as such as they elute early. Our goal was to get sufficient separation of the S8 peak from the polar interferences that elute starting immediately after the dead volume (DV) time. When the column is run at 95:5 MeOH:H_2O mobile phase, the S8 elutes with an acceptable peak shape and capacity factor (4.4) but is still in the tail of the polar interferences eluting prior, and the baseline is elevated by about 3.5 mAU relative to the start of the run (Figure 9a). At 90:10 MeOH:H_2O mobile phase, the S8 has separated further from the polar interference, with a baseline elevation above the injection baseline of about 2.3 mAU (Figure 9b). The capacity factor here is now 7.4, which is considered quite high for an elution of just one compound, and we see there is observable peak broadening due to the higher column residence time. For this reason, an even longer elution time is not preferred.

We noted from the full-scale chromatograms that the DV peak (containing much of the polar interference as well as the injection solvent, acetone) has a height of about 650 mAU. Therefore, it may be considered that under either of the mobile phase running conditions, at the time the S8 peak elutes the absorbance from the interference peak is >99% returned to baseline (Figures 9c and 9d). From the chromatograms with the y-axis scaled to 200 mAU, which is more representative of the full extent of the interference peak, it is more apparent that the S8 peak is not significantly impacted by the interference peak. The peak separation and resolution are better demonstrated with samples spiked at 100 µg/mL S8 (Figures 10a and 10b).

To achieve 100% return to baseline would require either a very long residence time for S8, resulting in unacceptable peak broadening, or a 15 cm column, which would result in an approximate 50% increase in retention time, although with less peak broadening than would be seen on the 10 cm column, and a comparable increase in cycle time.

We believe these elution conditions are an appropriate compromise between adequate separation from the interference peak, reasonable cycle time (sample throughput) and adequate accuracy and precision of the S8 peak.

Acetone Extracts

Figure 9a. HPLC chromatogram for the lodgepole pine leaf litter spiked at 20 µg/g and extracted with acetone. Extract concentration 2 µg/mL S8, mobile phase 95:5 MeOH:H_2O. Note: expanded y-axis, peak area 0.248.

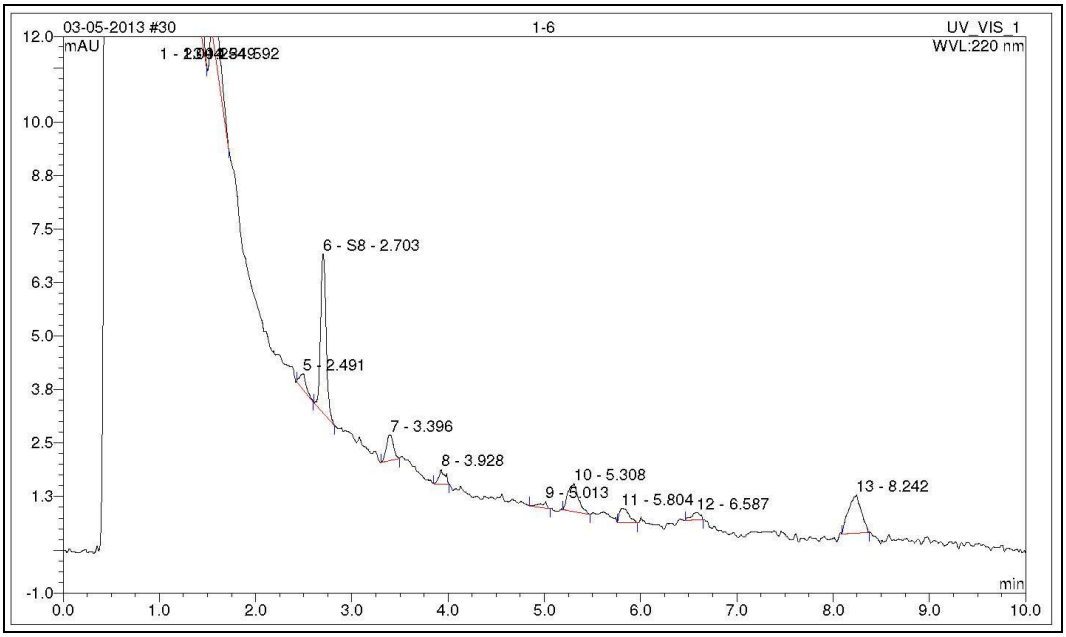


Figure 9b. HPLC chromatogram for the lodgepole pine leaf litter spiked at 20 µg/g and extracted with acetone. Extract concentration 2 µg/mL S8, mobile phase 90:10 MeOH:H2O. Note the improved separation for S8 peak, expanded y-axis, peak area 0.280.

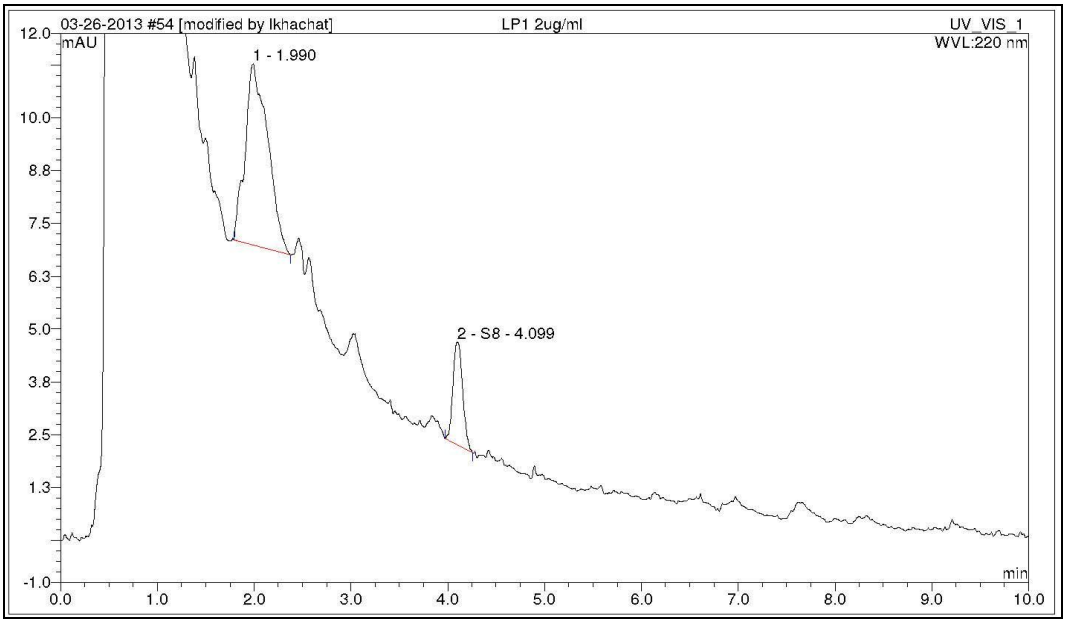


Figure 9c. HPLC chromatogram for the lodgepole pine leaf litter spiked at 20 µg/g and extracted with acetone. Extract concentration 2 µg/mL S8, mobile phase 90:10 MeOH:H2O. Note: y-axis rescaled to 200 mAU, peak area 0.280.

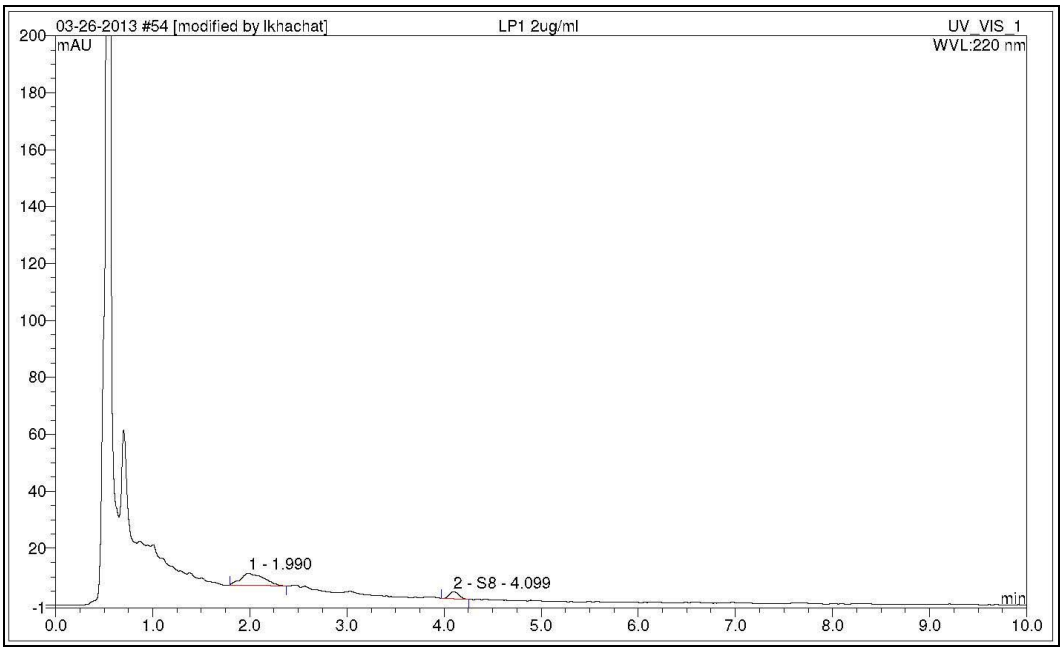


Figure 9d. HPLC chromatogram for the lodgepole pine leaf litter spiked at 20 µg/g and extracted with acetone. Extract concentration 2 µg/mL S8, mobile phase 90:10 MeOH:H_2O. Note: y-axis full scale at 700 mAU, peak area 0.280.

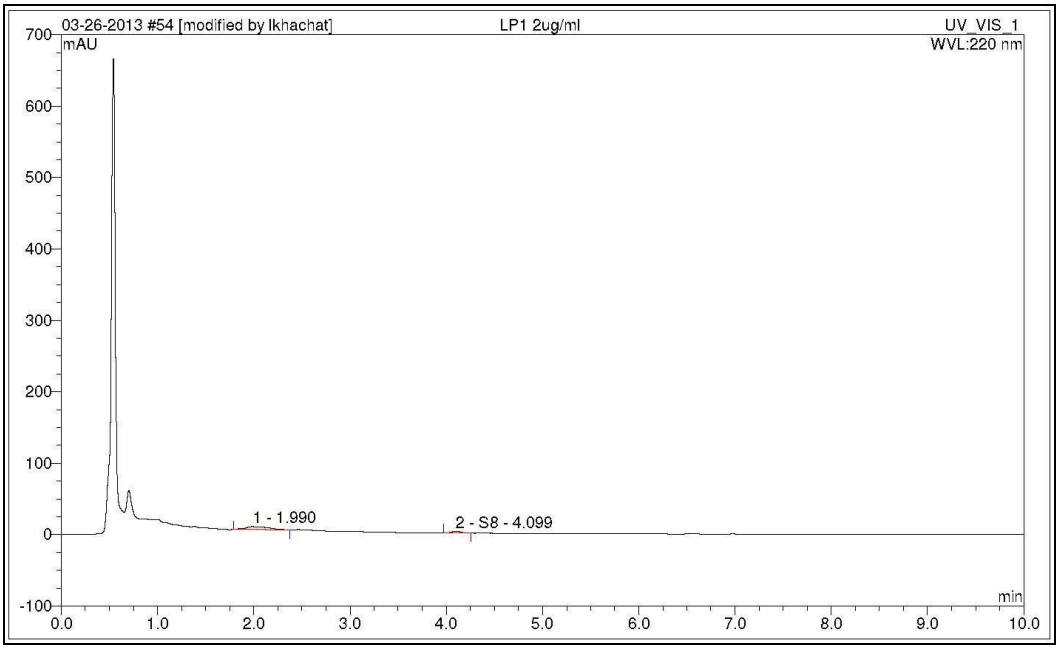


Figure 10a. HPLC chromatogram for the lodgepole pine leaf litter spiked at 1,000 µg/g S8. Extract concentration 100 µg/mL S8, mobile phase 90:10 MeOH:H_2O. Note: y-axis rescaled to 190 mAU.

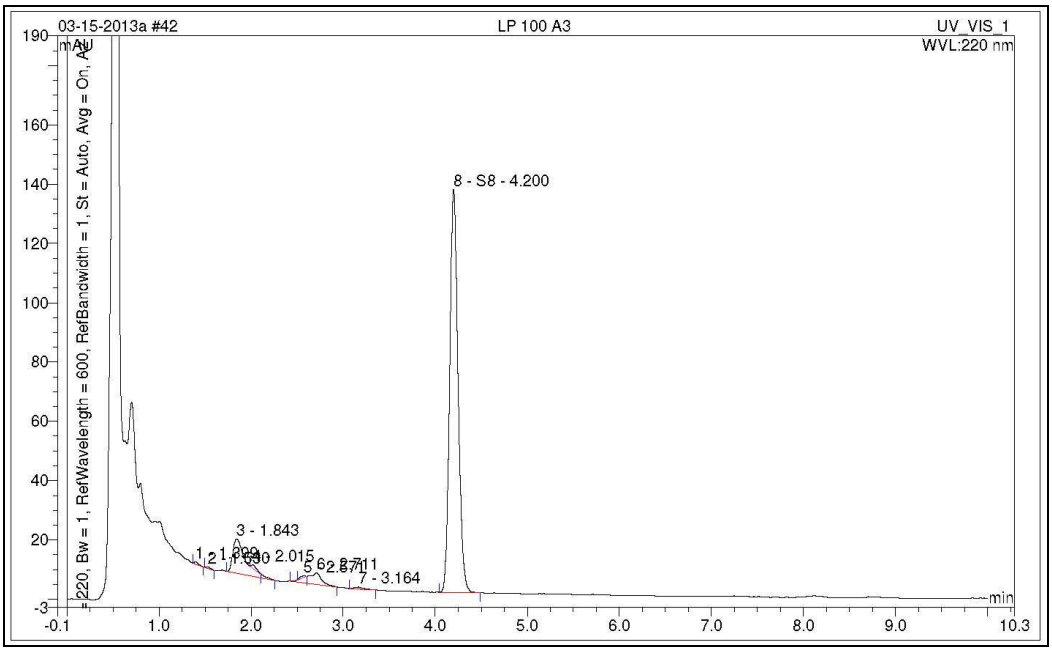
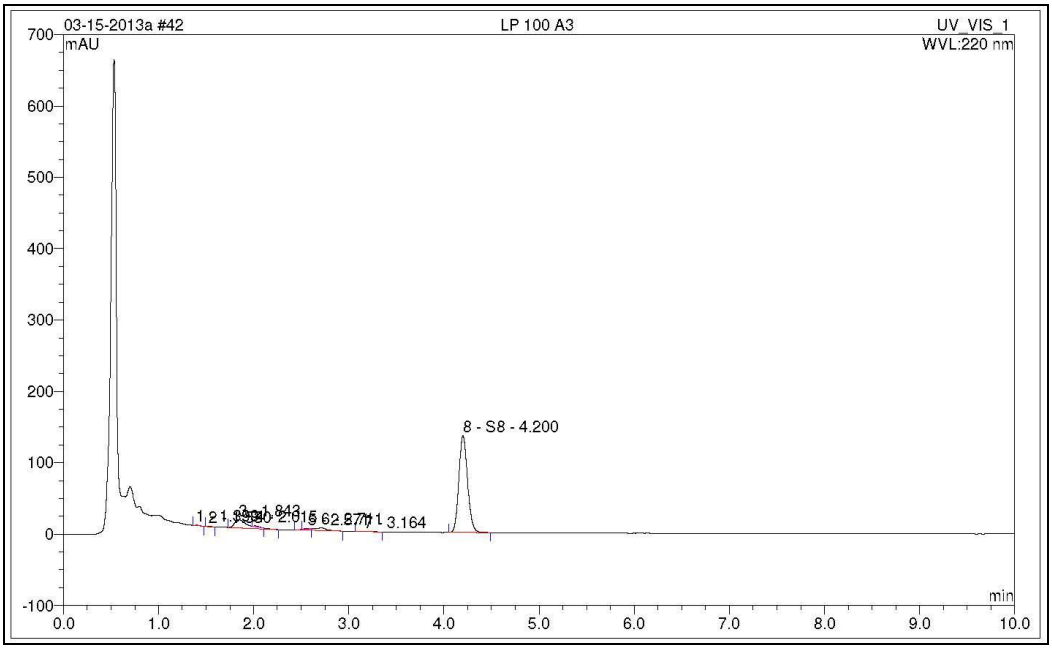


Figure 10b. HPLC chromatogram for the lodgepole pine litter spiked at 1,000 µg/g S8. Extract concentration 100 µg/mL S8, mobile phase 90:10 MeOH:H_2O. Note: y-axis full scale at 700 mAU.



DCM Extracts

Initial investigations determined the solubility of elemental sulphur in DCM to be about ten times greater than in acetone. DCM extracts were diluted 10x with acetone before HPLC analysis in order to prevent damage to the needle seats and extend the linear range of the method by an order of magnitude. Interestingly, the dilution procedure reduces the impact of the interference, as shown in the chromatograms below (Figures 11a and 11b) where an equivalent amount of S8 was injected, and S8 peak areas here and for the 2 µg/mL acetone injection (Figures 9b to 9d) are equivalent.

Figure 11a. HPLC chromatogram for lodgepole pine leaf litter spiked at 200 µg/g, DCM extract concentration 20 µg/mL S8, 2 µg/mL injected, mobile phase 90:10 MeOH:H_2O, peak area 0.268.

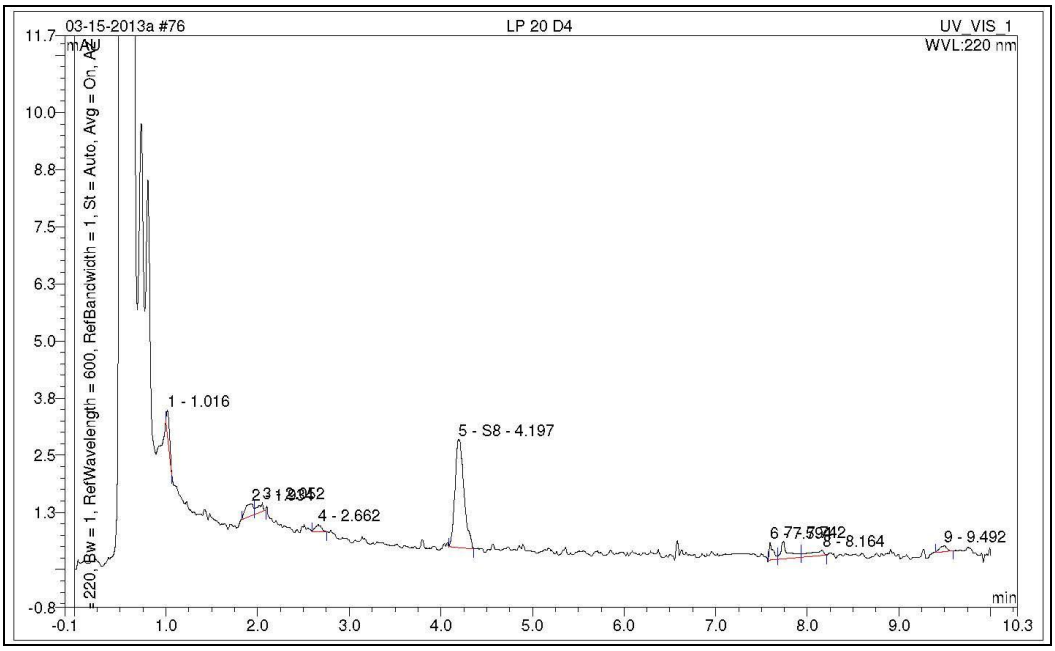
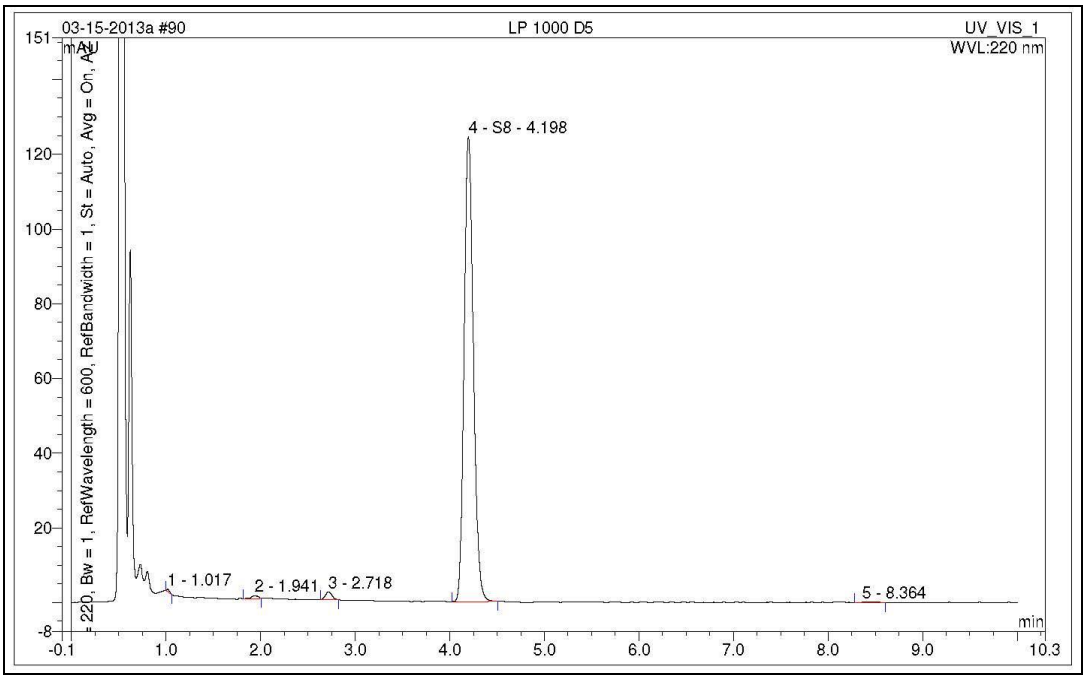


Figure 11b. HPLC chromatogram for the DCM extract from lodgepole pine leaf litter spiked at 10,000 µg/g, extract concentration 1,000 µg/mL S8, 100 µg/mL injected, mobile phase 90:10 MeOH:H_2O.



3.1.3 Instrument Parameters

The final instrument conditions were:

- Column: C18, 100 mm x 4.6 mm ID, 3.5 µm particle size, or equivalent;
- Guard column: C 18, 10 mm x 4.6 mm ID, 5 µm particle size, or equivalent (recommended for leaf litter soils);
- Column flow: 2.3 mL/min;
- Mobile phase: 90:10 MeOH:H_2O (v:v);
- Wavelength: 220 nm;
- Column temp.: 45°C; and
- Injection volume: 10 µL.

Acetone extracts are injected neat. DCM extracts are injected after 10x dilution with acetone.

Note 1: Diluting DCM extracts less than 10x is not recommended unless a Teflon® needle seat is employed. We observed near immediate deterioration of a standard needle seat with a 5x dilution of DCM extracts.

Note 2: Column contamination due to interferences from the lodgepole pine leaf litter extracts was a significant problem. We finally determined that a C18 guard cartridge is the simplest solution to this problem. If a high proportion of leaf litter samples is analysed,

the guard cartridge must be replaced every 20-100 injections (*i.e.,* daily). For minor contamination that still makes it past the guard cartridge, cleaning the analytical column with acetonitrile is sufficient. We sourced an inexpensive cartridge and holder for this purpose.

3.2 Method Validation

Government of Alberta requires a method that has a low detection limit to monitor levels in background and slightly impacted soils, as well as an alternative method for determining high levels of elemental sulphur in heavily contaminated sites and elemental sulphur-containing waste materials. Thus, two method validations are included. An acetone extract is proposed for the former soils because the extract can be run undiluted on the HPLC, and a DCM extract for the latter due to a much higher S8 solubility.

Method Validation includes:

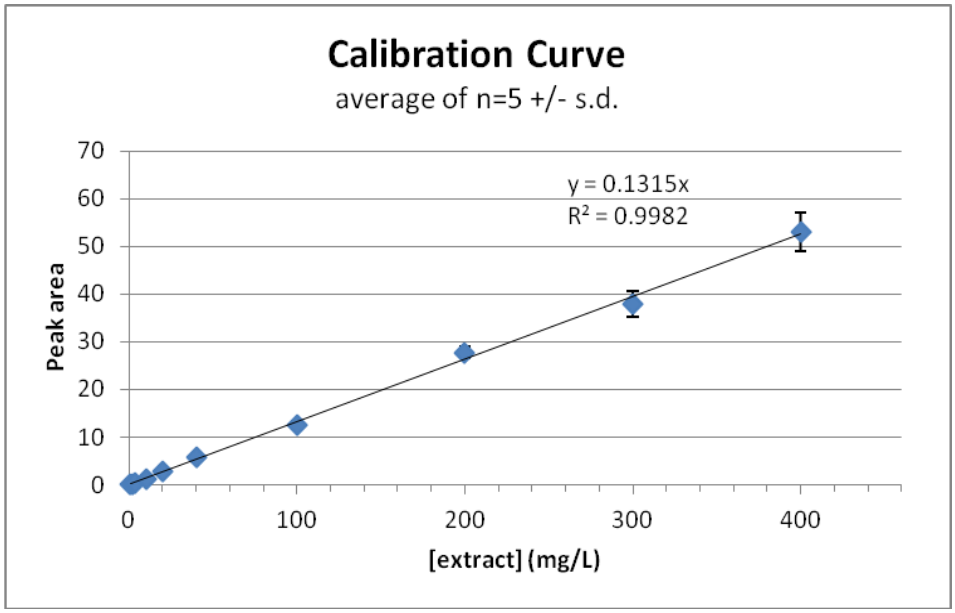
- Calibration and linear range;
- Method working range;
- Method detection limit;
- Method blank;
- Precision and accuracy;
- Selectivity;
- Robustness;
- Second source verification; and
- Certified reference material (CRM) analysis.

3.2.1 Calibration of Linear Range

Experiments showed that a linear calibration equation, y = mx + b with 1/x weighting, provides more accurate data at the low end of the curve. The calibration curve shows good linearity up to 400 µg/mL in the extract as injected (Figure 12). Using routine 2 g dried and ground sample and 20 mL solvent, the linear range can accommodate soils with elemental sulphur concentrations of up to 4,000 µg/g with the acetone extraction and 40,000 µg/g (or 4%) with the DCM extraction (including the 10x extract dilution).

Note: Acetone has significant volatility; therefore, good seals are required on autosampler vials for both calibration standards and extracts. Acetone was selected for calibration standard preparation due to the significantly better solubility of S8 in acetone relative to methanol and acetonitrile. The chlorinated solvents, in which S8 has high solubility, are not compatible with the mobile phase or standard reversed phase system seals because they attack the standard seals of the HPLC pump.

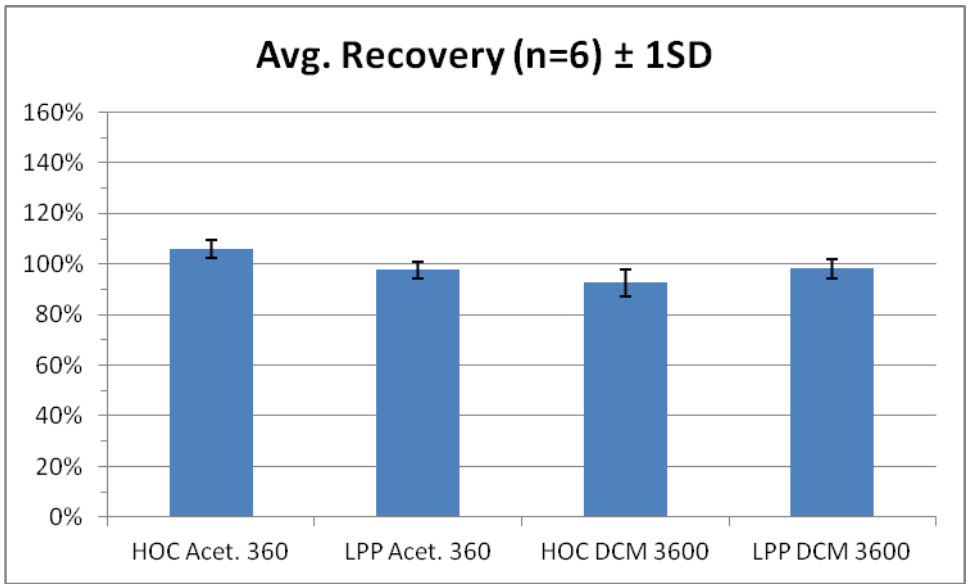
Figure 12. Linear Range of the HPLC Method



3.2.2 Method Working Range

The initial investigations of elemental sulphur solubility plus high level spikes in the method validation showed effective extraction up to 400 µg/mL in an acetone extract and 6,500 µg/mL in a DCM extract (assuming a 10:1 solvent:soil ratio). Any extracts with values above these levels must be re-extracted with a higher solvent:soil ratio. Also, any extracts with concentrations above the linear range of 400 µg/mL must be diluted into range. 400 µg/mL corresponds to 4,000 µg/g in a soil sample extracted with acetone, and 40,000 µg/g in a soil sample extracted with DCM as a 10× dilution step for DCM extracts is incorporated in the protocol. The recoveries for spikes at 90% of the linear range were between 88 and 108%. The values are shown in Figure 13 below.

Figure 13. Recovery of added elemental sulphur by the acetone-HPLC, and DCM-HPLC methods. (Note: HOC is high organic clay, and LPP is lodgepole pine leaf litter; 360 and 3,600 are the concentrations in the extract in µg/mL.)



3.2.3 Method Detection Limits (MDL)

Method detection limits are determined as per EPA 40 CFR Part 136 Appendix B and CCME[4] by the analysis of low level spiked samples carried through the entire analytical process. The MDL is defined as the standard deviation of the low level replicates multiplied by the *t* statistic for a one-tailed test at a 0.01 confidence level for n-1 degrees of freedom (*t* = 2.998 for n = 8). The spiking level must be between 1 and 10 times the calculated MDL. The MDL is intended to restrict the chance of false positives to 1% when there is no analyte present in the sample.

Eight 2 g replicates were extracted with 20 mL of solvent. DCM extracts were diluted an additional 10x prior to analysis. The results are presented in Table 5. Note that MDLs are normally determined in a single run using a clean matrix such as Ottawa sand. By using the two most difficult matrices, lodgepole pine leaf litter and high organic clay and analyzing on two different days, these MDLs must be considered conservative but reflect performance under routine operating conditions.

[4] Guidance Manual on Sampling, Analysis and Data Management, Volume Four, Updated Compendium of Analytical Methods for Contaminated Sites Section 6.3

Table 5. Method Detection Limit of the Acetone-HPLC and DCM-HPLC Method

µg/g Elemental Sulphur			
Acetone Extract	**Day 1**	**Day 2**	**Average**
Lodgepole Pine	6.4	5.4	6.8
High Organic Clay	6.2	9.3	
DCM Extract	**Day 1**	**Day 2**	**Average**
Lodgepole Pine	19	44	33.5
High Organic Clay	35	36	

3.2.4 Method Blank

Unspiked samples of Ottawa sand, leaf litters, and clays were analyzed. All values were non-detect except the high organic clay, which actually does contain 8 µg/g S8. The method contributes no measurable background.

3.2.5 Precision and Accuracy, Robustness

Low, mid and high level spiked replicates of lodgepole pine leaf litter and high organic clay extracted with acetone and DCM were analyzed on two separate days. Low level spikes were prepared by spiking 2 g aliquots of soil using a concentrated solution of S8 in DCM. The spiked samples were allowed to evaporate overnight in a fume hood prior to extraction. High level spikes were prepared by weighing the appropriate amount of S8 into a vial containing 2 g of sample. The soil-S8 mixtures were homogenized by tumbling prior to extraction. Both the sample preparation and analysis involved two analysts to demonstrate robustness. All data are included in the appended Master Data Table. Data are summarized in Table 6.

All recoveries are well within acceptance limits. The low level acetone spike of 20 µg/g is only 3x MDL. Maxxam corporate criteria at this level are: accuracy ± 50%, precision ± 20%. The high level recoveries (averaging 99%) validate the extraction efficiency at high concentrations of S8. Overall average recovery is 99.1% with an RSD of 5.3%.

Table 6. Average Percent Recovery and Relative Standard Deviation (RSD) of the Acetone-HPLC and DCM-HPLC Methods

Sample	Spike level (µg/g)	Average Recovery	RSD	Sample Size (n)
Acetone Extract				
Lodgepole Pine	20	118%	9.2%	8
High Organic Clay	20	113%	11.5%	8
Lodgepole Pine	200	101%	9.5%	8
High Organic Clay	200	105%	3.2%	8
Lodgepole Pine	1,000	84%	1.4%	6
High Organic Clay	1,000	90%	0.8%	6
Lodgepole Pine	3,600	98%	3.4%	6
High Organic Clay	3,600	106%	3.6%	6
DCM Extract				
Lodgepole Pine	200	94%	4.5%	8
High Organic Clay	200	89%	4.1%	9
Lodgepole Pine	10,000	101%	6.6%	6
High Organic Clay	10,000	98%	7.1%	6
Lodgepole Pine	36,000	98%	4.3%	5
High Organic Clay	36,000	93%	5.5%	6
	Overall Average	**99.1%**	**5.3%**	

3.2.6 Selectivity

Selectivity was discussed in detail in Section 3.1. The S8 peak is separated from interference peaks. The chromatograms of high matrix samples show non-detect results at the S8 elution time. Extraction efficiencies are good. In short, we have found nothing to indicate that the method will generate either false positives or false negatives.

3.2.7 Second Source Verification

A standard obtained from a second source was analyzed to verify the purity of the primary standard. Eight replicates prepared at 20 µg/mL were analyzed at regular intervals throughout a

100-sample run. The data are excellent: 98% recovery with an RSD of 3.5%. Recoveries are tabulated in Table 7.

Table 7. Second Source Verification Results

Replicate	Spike Recovery (µg/mL)
ICV-1	19.14
ICV-2	18.53
ICV-3	19.27
ICV-4	29.08*
ICV-5	19.47
ICV-6	20.78
ICV-7	19.87
ICV-8	19.55
Average (excluding outlier)	**19.52**
Theoretical	**20**
% Recovery	**98%**
% RSD (excluding outlier)	**3.5%**

*Outlier. Peak badly tailed. Runs before and after were normal.

3.2.8 Certified Reference Material (CRM)

After an extensive search, a CRM was found. None of the standard suppliers of CRM for environmental analysis had a soil CRM with elemental sulphur. We identified a CRM for the mining industry, a mine-tailing sample, Canadian Certified Reference Materials Project, Sulphide Ore Mill Tailings Reference Materials, RTS-1[5]. The matrix is essentially finely ground rock. The Certificate of Analysis is provided in Appendix 2. The value for S8 composition given, 0.50% ± 0.16%, is informational, not certified or provisional, but this was the only suitable matrix we could identify.

The CRM was used to verify the accuracy of the entire process. Neither the sample preparation technician nor the analyst knew the concentration of S8 in the CRM. CRM results are tabulated in Table 8.

[5] Canmet, 555 Booth Street, Ottawa, ON, K1A 0G1

Table 8. Sulphur Recovery in Certified Reference Material

Replicate	Measured Concentration (µg/g)
rep 1	3,237
rep 2	3,361
rep 3	3,060
rep 4	3,075
rep 5	3,211
Average	**3,189**
Standard Deviation	**125**
%RSD	**3.9%**
Average in %	**0.32% ± 0.01%**

The Certificate of Analysis for RTS-1 provides only an informational value for elemental sulphur: 0.50% ± 0.16%. Our result at 0.32% ± 0.01% is just below the lower end of the range, but since it is an informational value, it is acceptable at this time.

The CRM has been included in our round robin. There would be at least four different methods used. It will be interesting to see if the results of our round robin for this material will be sufficient for Natural Resources Canada to update their information on RTS-1 to a certified value.

4 Summary

The method has passed all validation criteria. The completed SOP is appended as Attachment A.

A round robin project on this SOP and other related analytical methods for elemental sulphur was conducted with a variety of sample matrices and levels of spikes. The detailed approaches and results are provided as Attachment B.

5 References

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6 Appendices

Appendix 1: Master Data Table

Appendix 2: Certificate of Analysis

7 Attachments

Attachment A: Standard Operating Procedure for the Analysis of Elemental Sulphur in Alberta Soils by High Performance Liquid Chromatography

Attachment B: Elemental Sulphur Methods Round Robin

8 Acknowledgements

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Appendix 1 Master Data Table

Method Blank

Sample #	Sample name	Extraction solvent	S expected extract conc. (mg/L)	S conc. (mg/L) 2013/03/04	S conc. (mg/L) 2013/03/04 with blank correction	Recovery %	S conc. (mg/L) 2013/03/05	S conc. (mg/L) 2013/03/05 with blank correction	Recovery %		
7-1	Ottawa sand	Acetone	-	ND	ND	NA	ND	ND	NA		
7-2	Ottawa sand	Acetone	-	ND	ND	NA	ND	ND	NA		
8	Non-spiked lodgepole	Acetone	-	ND	NA	NA	ND	NA	NA		
9	Non-spiked clay/org 9%	Acetone	-	0.86	NA	NA	0.81	NA	NA	Average (mg/L)	0.84

ND = Not detected

NA = Not applicable

Precision and Accuracy, MDL
Acetone

Sample #	Sample name	Extraction solvent	S expected extract conc. (mg/L)	S conc. (mg/L) Rep 1	S conc. (mg/L) Rep 1 with blank correction	Recovery %	S conc. (mg/L) Rep 2	S conc. (mg/L) Rep 2 with blank correction	Recovery %	Average S8 conc. in extract (mg/L)	Average S8 conc. In soil (mg/kg)	Average Recovery	
Edmonton Extracts					**04-Mar**			**05-Mar**					
1-1	Lodgepole	Acetone	2	2.18	2.18	109	2.31	2.31	116	2.25	22.5	112.3%	Precision
1-2	Lodgepole	Acetone	2	2.39	2.39	120	2.48	2.48	124	2.44	24.4	121.8%	9.2%
1-3	Lodgepole	Acetone	2	1.96	1.96	98	2.36	2.36	118	2.16	21.6	108.0%	Accuracy
1-4	Lodgepole	Acetone	2	2.44	2.44	122	2.74	2.74	137	2.59	25.9	129.5%	17.5%
1-5	Lodgepole	Acetone	2	2.51	2.51	126	2.59	2.59	130	2.55	25.5	127.5%	Count
1-6	Lodgepole	Acetone	2	1.93	1.93	97	2.20	2.20	110	2.07	20.7	103.3%	8
1-7	Lodgepole	Acetone	2	2.18	2.18	109	2.64	2.64	132	2.41	24.1	120.5%	Avg. Recovery %
1-8	Lodgepole	Acetone	2	2.31	2.31	116	2.39	2.39	120	2.35	23.5	117.5%	117.5%
3-1	clay/org 9%	Acetone	2	3.36	3.36	168	3.43	3.43	171	3.39	33.9	169.7%	Precision
3-2	clay/org 9%	Acetone	2	2.77	2.77	139	2.91	2.91	146	2.84	28.4	142.1%	11.5%
3-3	clay/org 9%	Acetone	2	3.18	3.18	159	2.88	2.88	144	3.03	30.3	151.5%	Accuracy
3-4	clay/org 9%	Acetone	2	3.05	3.05	153	2.93	2.93	146	2.99	29.9	149.5%	54.5%
3-5	clay/org 9%	Acetone	2	3.17	3.17	159	3.45	3.45	172	3.31	33.1	165.4%	Count
3-6	clay/org 9%	Acetone	2	3.11	3.11	156	3.62	3.62	181	3.37	33.7	168.3%	8
3-7	clay/org 9%	Acetone	2	3.02	3.02	151	2.88	2.88	144	2.95	29.5	147.5%	Avg. Recovery %
3-8	clay/org 9%	Acetone	2	2.75	2.75	138	2.94	2.94	147	2.84	28.4	142.2%	154.5%
2-1	Lodgepole	Acetone	20	19.83	19.83	99	20.68	20.68	103	20.25	202.5	101.3%	Precision
2-2	Lodgepole	Acetone	20	21.04	21.04	105	20.27	20.27	101	20.65	206.5	103.3%	9.5%
2-3	Lodgepole	Acetone	20	16.10	16.10	80	16.93	16.93	85	16.52	165.2	82.6%	Accuracy
2-4	Lodgepole	Acetone	20	17.68	17.68	88	22.30	22.30	111	19.99	199.9	100.0%	1.4%
2-5	Lodgepole	Acetone	20	21.40	21.40	107	24.49	24.49	122	22.95	229.5	114.7%	Count
2-6	Lodgepole	Acetone	20	19.50	19.50	98	24.70	24.70	123	22.10	221.0	110.5%	8
2-7	Lodgepole	Acetone	20	20.48	20.48	102	19.88	19.88	99	20.18	201.8	100.9%	Avg. Recovery %
2-8	Lodgepole	Acetone	20	16.66	16.66	83	22.46	22.46	112	19.56	195.6	97.8%	101.4%
4-1	clay/org 9%	Acetone	20	19.88	19.88	99	25.78	25.78	129	22.83	228.3	114.1%	Precision
4-2	clay/org 9%	Acetone	20	23.39	23.39	117	19.63	19.63	98	21.51	215.1	107.5%	3.2%
4-3	clay/org 9%	Acetone	20	22.47	22.47	112	20.51	20.51	103	21.49	214.9	107.4%	Accuracy
4-4	clay/org 9%	Acetone	20	21.33	21.33	107	22.38	22.38	112	21.86	218.6	109.3%	9.6%
4-5	clay/org 9%	Acetone	20	20.65	20.65	103	23.04	23.04	115	21.85	218.5	109.2%	Count
4-6	clay/org 9%	Acetone	20	21.20	21.20	106	24.74	24.74	124	22.97	229.7	114.9%	8
4-7	clay/org 9%	Acetone	20	21.12	21.12	106	22.28	22.28	111	21.70	217.0	108.5%	Avg. Recovery %
4-8	clay/org 9%	Acetone	20	23.33	23.33	117	19.09	19.09	95	21.21	212.1	106.0%	109.6%

Precision and Accuracy, MDL

Acetone

Sample #	Sample name	Extraction solvent	S expected extract conc. (mg/L)	S conc. (mg/L) Rep 1	S conc. (mg/L) Rep 1 with blank correction	Recovery %	S conc. (mg/L) Rep 2	S conc. (mg/L) Rep 2 with blank correction	Recovery %	Average S8 conc. in extract (mg/L)	Average S8 conc. In soil (mg/kg)	Average Recovery		
Mississauga Extracts					**15-Mar**			**18-Mar**						
2-1	Lodgepole	Acetone	100	84.28	84.28	84	84.84	84.84	85	84.56	845.6	84.6%	Precision	
2-2	Lodgepole	Acetone	100	84.37	84.37	84	80.69	80.69	81	82.53	825.3	82.5%	1.4%	
2-3	Lodgepole	Acetone	100	83.67	83.67	84	81.69	81.69	82	82.68	826.8	82.7%	Accuracy	
2-4	Lodgepole	Acetone	100	84.00	84.00	84	85.90	85.90	86	84.95	849.5	84.9%	15.7%	
2-5	Lodgepole	Acetone	100	87.22	87.22	87	84.35	84.35	84	85.79	857.9	85.8%	Avg. Recovery %	Count
2-6	Lodgepole	Acetone	100	86.44	86.44	86	83.96	83.96	84	85.20	852.0	85.2%	84.3%	6
1-1	clay/org 9%	Acetone	100	90.18	90.18	90	92.23	92.23	92	91.21	912.1	91.2%	Precision	
1-2	clay/org 9%	Acetone	100	91.69	91.69	92	88.74	88.74	89	90.21	902.1	90.2%	0.8%	
1-3	clay/org 9%	Acetone	100	91.10	91.10	91	92.22	92.22	92	91.66	916.6	91.7%	Accuracy	
1-4	clay/org 9%	Acetone	100	90.05	90.05	90	91.46	91.46	91	90.76	907.6	90.8%	9.3%	
1-5	clay/org 9%	Acetone	100	91.46	91.46	91	90.22	90.22	90	90.84	908.4	90.8%	Avg. Recovery %	Count
1-6	clay/org 9%	Acetone	100	90.13	90.13	90	88.78	88.78	89	89.45	894.5	89.5%	90.7%	6
					26-Mar			**27-Mar**						
LP1-High Lev.	Lodgepole	Acetone	360	357.58	357.58	99	390.4255	390.43	108	374.00	3740.0	103.9%	Precision	
LP2-High Lev.	Lodgepole	Acetone	360	340.50	340.50	95	351.1404	351.14	98	345.82	3458.2	96.1%	3.4%	
LP3-High Lev.	Lodgepole	Acetone	360	343.37	343.37	95	353.2575	353.26	98	348.31	3483.1	96.8%	Accuracy	
LP4-High Lev.	Lodgepole	Acetone	360	341.24	341.24	95	368.4424	368.44	102	354.84	3548.4	98.6%	2.3%	
LP5-High Lev.	Lodgepole	Acetone	360	329.81	329.81	92	368.469	368.47	102	349.14	3491.4	97.0%	Avg. Recovery %	Count
LP6-High Lev.	Lodgepole	Acetone	360	330.92	330.92	92	344.8833	344.88	96	337.90	3379.0	93.9%	97.7%	6
HOC1-High Lev.	clay/org 9%	Acetone	360	366.91	366.91	102	394.95	394.95	110	380.93	3809.3	105.8%	Precision	
HOC2-High Lev.	clay/org 9%	Acetone	360	396.27	396.27	110	379.21	379.21	105	387.74	3877.4	107.7%	3.6%	
HOC3-High Lev.	clay/org 9%	Acetone	360	395.70	395.70	110	393.91	393.91	109	394.80	3948.0	109.7%	Accuracy	
HOC4-High Lev.	clay/org 9%	Acetone	360	393.26	393.26	109	400.07	400.07	111	396.67	3966.7	110.2%	6.4%	
HOC5-High Lev.	clay/org 9%	Acetone	360	365.09	365.09	101	384.41	384.41	107	374.75	3747.5	104.1%	Avg. Recovery %	Count
HOC6-High Lev.	clay/org 9%	Acetone	360	326.65	326.65	91	399.51	399.51	111	363.08	3630.8	100.9%	106.4%	6

Precision and Accuracy, MDL

DCM

Sample #	Sample name	Extraction solvent	S expected extract conc. (mg/L)	S conc. (mg/L) Rep 1	S conc. (mg/L) Rep 1 with blank correction	Recovery %	S conc. (mg/L) Rep 2	S conc. (mg/L) Rep 2 with blank correction	Recovery %	Average S8 conc. in extract (mg/L)	Average S8 conc. In soil (mg/kg)	Average Recovery	
Mississauga Extracts					**15-Mar**			**18-Mar**					
5-1	Lodgepole	DCM	20	17.75	17.75	89	18.47	18.47	92	18.11	181.1	90.6%	Precision
5-2	Lodgepole	DCM	20	19.38	19.38	97	20.78	20.78	104	20.08	200.8	100.4%	4.5%
5-3	Lodgepole	DCM	20	18.97	18.97	95	18.13	18.13	91	18.55	185.5	92.8%	Accuracy
5-4	Lodgepole	DCM	20	18.60	18.60	93	16.23	16.23	81	17.42	174.2	87.1%	6.5%
5-5	Lodgepole	DCM	20	18.35	18.35	92	17.20	17.20	86	17.77	177.7	88.9%	Avg. Recovery %
5-6	Lodgepole	DCM	20	18.80	18.80	94	20.20	20.20	101	19.50	195.0	97.5%	93.5%
5-7	Lodgepole	DCM	20	19.09	19.09	95	18.99	18.99	95	19.04	190.4	95.2%	Count
5-8	Lodgepole	DCM	20	19.83	19.83	99	18.43	18.43	92	19.13	191.3	95.6%	8
3-1	clay/org 9%	DCM	20	20.42	20.42	102	19.26	19.26	96	19.84	198.4	99.2%	Precision
3-2	clay/org 9%	DCM	20	19.06	19.06	95	18.63	18.63	93	18.84	188.4	94.2%	4.1%
3-3	clay/org 9%	DCM	20	18.10	18.10	90	17.76	17.76	89	17.93	179.3	89.6%	Accuracy
3-4	clay/org 9%	DCM	20	21.02	21.02	105	18.49	18.49	92	19.76	197.6	98.8%	6.6%
3-5	clay/org 9%	DCM	20	18.25	18.25	91	18.22	18.22	91	18.23	182.3	91.2%	Avg. Recovery %
3-6	clay/org 9%	DCM	20	20.18	20.18	101	16.21	16.21	81	18.20	182.0	91.0%	93.4%
3-7	clay/org 9%	DCM	20	18.54	18.54	93	20.05	20.05	100	19.30	193.0	96.5%	Count
3-8	clay/org 9%	DCM	20	17.94	17.94	90	16.90	16.90	84	17.42	174.2	87.1%	9
3-9	clay/org 9%	DCM	20	20.07	20.07	100	17.30	17.30	87	18.69	186.9	93.4%	

Precision and Accuracy, MDL
DCM

Sample #	Sample name	Extraction solvent	S expected extract conc. (mg/L)	S conc. (mg/L) Rep 1	S conc. (mg/L) Rep 1 with blank correction	Recovery %	S conc. (mg/L) Rep 2	S conc. (mg/L) Rep 2 with blank correction	Recovery %	Average S8 conc. in extract (mg/L)	Average S8 conc. In soil (mg/kg)	Average Recovery		
Mississauga Extracts					**26-Mar**			**27-Mar**						
LP1-Mid Lev.	Lodgepole	DCM	1000	984.694	984.69	98	1059.84	1059.84	106	1022.27	10222.7	102.2%	Precision	
LP2-Mid Lev.	Lodgepole	DCM	1000	888.3687	888.37	89	1128.82	1128.82	113	1008.59	10085.9	100.9%	6.6%	
LP3-Mid Lev.	Lodgepole	DCM	1000	1111.773	1111.77	111	1119.56	1119.56	112	1115.67	11156.7	111.6%	Accuracy	
LP4-Mid Lev.	Lodgepole	DCM	1000	836.8425	836.84	84	995.80	995.80	100	916.32	9163.2	91.6%	1.1%	
LP5-Mid Lev.	Lodgepole	DCM	1000	887.3657	887.37	89	1056.96	1056.96	106	972.16	9721.6	97.2%	Avg. Recovery %	Count
LP6-Mid Lev.	Lodgepole	DCM	1000	996.9422	996.94	100	1062.91	1062.91	106	1029.93	10299.3	103.0%	101.1%	6
HOC1-Mid Lev.	clay/org 9%	DCM	1000	878.09	878.09	88	802.15	802.15	80	840.12	8401.2	84.0%	Precision	
HOC2-Mid Lev.	clay/org 9%	DCM	1000	848.67	848.67	85	1098.33	1098.33	110	973.50	9735.0	97.3%	7.1%	
HOC3-Mid Lev.	clay/org 9%	DCM	1000	887.09	887.09	89	1106.84	1106.84	111	996.96	9969.6	99.7%	Accuracy	
HOC4-Mid Lev.	clay/org 9%	DCM	1000	914.24	914.24	91	1092.23	1092.23	109	1003.23	10032.3	100.3%	2.2%	
HOC5-High Lev.	clay/org 9%	DCM	1000	970.06	970.06	97	1051.26	1051.26	105	1010.66	10106.6	101.1%	Avg. Recovery %	Count
HOC6-Mid Lev.	clay/org 9%	DCM	1000	1047.59	1047.59	105	1036.71	1036.71	104	1042.15	10421.5	104.2%	97.8%	6

					26-Mar			**27-Mar**						
LP1-High Lev.	Lodgepole	DCM	3600	3349.48	3349.48	93	3493.96	3493.96	97	3421.72	34217.2	95.0%	Precision	
LP2-High Lev.	Lodgepole	DCM	3600	3612.84	3612.84	100	2017.09	2017.09	56	*	-	*	4.3%	
LP3-High Lev.	Lodgepole	DCM	3600	3541.80	3541.80	98	3582.81	3582.81	100	3562.30	35623.0	99.0%	Accuracy	
LP4-High Lev.	Lodgepole	DCM	3600	3578.09	3578.09	99	3283.38	3283.38	91	3430.74	34307.4	95.3%	2.3%	
LP5-High Lev.	Lodgepole	DCM	3600	3929.76	3929.76	109	3606.04	3606.04	100	3767.90	37679.0	104.7%	Avg. Recovery %	Count
LP6-High Lev.	Lodgepole	DCM	3600	3650.82	3650.82	101	3160.41	3160.41	88	3405.62	34056.2	94.6%	97.7%	5
HOC1-High Lev.	clay/org 9%	DCM	3600	3595.72	3595.72	100	3468.36	3468.36	96	3532.04	35320.4	98.1%	Precision	
HOC2-High Lev.	clay/org 9%	DCM	3600	3011.81	3011.81	84	3044.31	3044.31	85	3028.06	30280.6	84.1%	5.5%	
HOC3-High Lev.	clay/org 9%	DCM	3600	3390.46	3390.46	94	3590.06	3590.06	100	3490.26	34902.6	97.0%	Accuracy	
HOC4-High Lev.	clay/org 9%	DCM	3600	3491.15	3491.15	97	3315.71	3315.71	92	3403.43	34034.3	94.5%	7.3%	
HOC5-High Lev.	clay/org 9%	DCM	3600	3174.57	3174.57	88	3161.50	3161.50	88	3168.03	31680.3	88.0%	Avg. Recovery %	Count
HOC6-High Lev.	clay/org 9%	DCM	3600	3475.32	3475.32	97	3308.51	3308.51	92	3391.91	33919.1	94.2%	92.7%	6

*Outlier - removed from calculation

Appendix 2 Certificate of Analysis

CCRMP
Canadian Certified Reference Materials Project

CANMET Mining and Mineral Sciences Laboratories
555 Booth Street, Ottawa, Ontario, Canada K1A 0G1
Tel.: (613) 995-4738, Fax: (613) 943-0573
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www.pcmrc.ca

Certificate of Analysis

First issued: 1990 Version: June 2009

RTS-1, RTS-2, RTS-3, RTS-4

SULPHIDE ORE MILL TAILINGS REFERENCE MATERIALS

Table 1a - Summary of Certified Values

	RTS-1	RTS-2	RTS-3	RTS-4
S_{total} %	1.66 ± 0.04	18.95 ± 0.37	9.98 ± 0.26	-
Si, %	19.89 ± 0.46	2.92 ± 0.18	15.99 ± 0.46	-
Ca, %	2.67 ± 0.09	0.53 ± 0.03	2.20 ± 0.09	0.327 ± 0.028
Al, %	4.26 ± 0.15	-	4.79 ± 0.17	-
Mg, %	2.67 ± 0.07	0.351 ± 0.012	2.45 ± 0.06	-
Cu, µg/g	595 ± 18	670 ± 32	2820 ± 90	-
Zn, µg/g	553 ± 31	117 ± 10	1850 ± 80	158 ± 14
Pb, µg/g	-	-	146 ± 20	-
Ni, µg/g	-	2430 ± 100	-	-
Co, µg/g	16.6 ± 3.9	-	260 ± 16	-
As, µg/g	-	-	9.1 ± 2.6	-

Natural Resources Canada Ressources naturelles Canada

Canada

Table 1b - Summary of Provisional Values

	RTS-1	RTS-2	RTS-3	RTS-4
S_{total} %	-	-	-	35.9 ± 1.2
$S_{sulphate}$ %	1.26 ± 0.05	3.87 ± 0.027	1.54 ± 0.12	0.27 ± 0.05
Fe, %	19.64 ± 0.71	37.4 ± 1.2	21.04 ± 0.66	56.7 ± 1.8
Si, %	-	-	-	0.998 ± 0.078
Al, %	-	0.83 ± 0.05	-	0.339 ± 0.030
Mg, %	-	-	-	0.179 ± 0.019
Cu, µg/g	-	-	-	280 ± 15
Pb, µg/g	105 ± 18	45 ± 21	-	60 ± 24
Ni, µg/g	22 ± 7	-	71 ± 13	7940 ± 360
Co, µg/g	-	72 ± 7	-	186 ± 21
As, µg/g	8.2 ± 1.6	6.3 ± 1.8	-	207 ± 44

Table 2 - Summary of Informational Values

	RTS-1	RTS-2	RTS-3	RTS-4
$S_{elemental}$ %(1)	0.50 ± 0.16	14.47 ± 0.14	2.81 ± 0.08	0.43 ± 0.03
$S_{sulphide}$ %(2)	0.0 ± 0.07	0.61 ± 0.40	5.63 ± 0.30	35.2 ± 1.2
Fe_{TITR}, %(3)	19.89 ± 0.26	37.90 ± 0.37	21.15 ± 0.15	56.64 ± 0.50
Mn, %	0.19	0.04	0.20	0.015
Na, %	0.50	0.22	0.51	0.07
K, %	0.52	0.12	0.35	0.04
Ti, %	0.40	0.16	0.32	0.08
P, %	0.06	0.02	0.05	0.02
CO_2, %	<0.1	<0.2	<0.2	<0.7
C_{total}, %	<0.9	<2	<0.9	<1.5
H_2O^-, %	1.5	1.7	1.8	0.16
Cd, µg/g	2	2	9.1 ± 1.6	5
Ba, µg/g	123	72	98	27
Bi, µg/g	81	3	100	3.3
Cr, µg/g	50	125	75	100
Se, µg/g	40	57	61	100
Sr, µg/g	60	30	40	12
Zr, µg/g	110	20	66	10
Ag, µg/g	<3	<2	<8	<2
Au, ng/g	262 ± 30	38 ± 10	235 ± 23	21 ± 9
Pt, ng/g	<70	217 ± 19	<70	55 ± 36
Pd, ng/g	<20	136 ± 16	<20	15

(1) CANMET values; two sets
(2) Computed by difference from CANMET values.
(3) Sets obtained by volumetric titration only.

RTS - 1, RTS - 2, RTS - 3, RTS - 4 page 2 of 3

Four sulphide ore tailings reference materials were prepared and characterized by the Canadian Certified Reference Materials Project (CCRMP) at the request of the Mine Environment Neutral Drainage Project (MEND) - an industry/government project on reactive acid tailings management.

The materials identified as RTS-1 and RTS-3 were prepared from samples drawn by Noranda Inc. from the oxidized (vadose) zone and the saturated ("unoxidized") zone of the Waite-Amulet tailings, located approximately 20 km north of Noranda, Quebec. RTS-2 is a product provided by INCO Ltd., Sudbury, Ontario, in response to a request for a low pyrrhotite (oxidized) material. RTS-4 is a pyrrhotite concentrate donated by Falconbridge Limited in response to the request for a high pyrrhotite (unoxidized) material from Sudbury.

PREPARATION

RTS-1, RTS-2, and RTS-3 were batch-dried on a steam pipe bed or low-temperature oven. Dried material was crushed in Denver rollers and milled in 30-kg batch lots in a vibration-energy mill. The materials were sieved to -200 mesh, blended in a 540-L conical blender, and bottled in 100-g units in laminated aluminum foil-mylar pouches.

RTS-4 could not be dried conventionally because of thermal decomposition. Instead, a laborious combination of vacuum filtration and acetone washing was employed to produce 27 kg of -200 mesh material. After blending, RTS-4 was bottled in 25-g units which were sealed in laminated aluminum foil-mylar pouches.

MEASUREMENT PROGRAM

A systematic sampling scheme was used to select samples for analytical measurements and homogeneity evaluation. Seventeen laboratories provided triplicate results on two bottles of each material for up to 30 constituents. Certified and provisional values ± 95 % confidence limits for 13 constituents are presented in Tables Ia and 1b, respectively. Informational values for some 20 other constituents are given in Table II.

CERTIFICATION HISTORY

RTS-1 to RTS-4 were first released in 1990. In 1996 the certificate was re-issued on the new CCRMP letterhead. In 2009 the certificate was re-issued with the certified and provisional values previously shown in Table 1 separated into two Tables, Ia and Ib respectively, for clarity. None of the values have changed since 1990.

LEGAL NOTICE

The Canadian Certified Reference Materials Project (CCRMP) has prepared these reference materials and evaluated the analytical data of the certification program to the best of its ability. The purchaser, by receipt hereof, releases and indemnifies CCRMP from and against all liability and costs arising out of the use of these materials and information.

REFERENCE

A CANMET-MMSL report, CCRMP 90-3E, describing the preparation and certification procedures for these reference materials is available at no charge upon request to:

CCRMP
CANMET (NRCan)
555 Booth Street
Ottawa, Ontario, Canada
K1A 0G1
Telephone: (613) 995-4738
Facsimile: (613) 943-0573
E-mail : ccrmp@nrcan.gc.ca

Attachment A. Standard Operating Procedure for the Analysis of Elemental Sulphur in Alberta Soils by High Performance Liquid Chromatography

Developed by Maxxam Analytics for Alberta Environment and Parks

June, 2015

Attachment A - Table Of Contents

1 Introduction

Elemental sulphur is one of the regulated substances in the Province of Alberta and analytical methods for its determination need to be further developed for monitoring and mitigating its impact on soils. Maxxam Analytics International Corporation was previously contracted by the former Department of Alberta Environment and Sustainable Resource Development (ESRD) to develop a High Performance Liquid Chromatography (HPLC) analytical method for quantitation of elemental sulphur in soils. This Standard Operating Procedure (SOP) is provided to ensure consistency in methodology and data quality objectives, following completion of the round robin by participating laboratories.

1.1 Scientific Principle

Sulphur is one of the non-metal elements that is abundantly available in the elemental form. It is an odourless, brittle, yellow solid that is easily oxidized or reduced, depending on its environment. Sulphur in the elemental form is used directly in the manufacture of pulp and paper, carbon disulfide, fertilizers, and rubber and other elastomers. It is used in industry in the form of sulphuric acid. Sulphur occurs naturally near volcanoes and hot springs as well as in natural gas and petroleum crudes. Elemental sulphur is slowly converted to sulfate in soil by the action of autotrophic bacteria, the most important of which belong to the genus *Thiobacillus*. Variability in elemental sulphur oxidation rates among soils is reported[6] to be related to the differences in the number of *Thiobacillus*. The oxidized sulphur slowly leaches from the soil as sulfate.

Elemental sulphur (S8) is insoluble in water and only slightly soluble in organic solvents such as ether, petroleum ether, toluene, chloroform, and alcohol.

In this method, after drying and grinding to < 2 mm, soil samples are extracted with either acetone or dichloromethane (DCM). These solvents effectively extract all forms of elemental sulphur from the soil matrix up to concentrations limited by the solubility of S8 in the solvent. The extract is separated from the soil residue and analyzed by High Performance Liquid Chromatography (HPLC) using a methanol-water mobile phase and UV detection at 220 nm.

2 Scope

2.1 Applicability

This method is suitable for determination of elemental sulphur in soils at concentrations from

[6] Havlin, J.L., Beaton, J.D., Tisdale S.L. and Nelson, W.L., 1999. Soil Fertility and Fertilizers – An Introduction to Nutrient Management. 6th ed., Prentice Hall Inc., New Jersey.

10 to 40,000 mg/kg. The range can be extended by dilution and/or extraction at a higher solvent to soil ratio.

3 Definitions and Acronyms

Continuing Calibration Verification (CCV): Usually the midpoint standard of the calibration curve, analyzed to verify the applicability of the calibration curve.

Initial Calibration Blank (ICB): Continuing Calibration Blank (CCB): Aliquots of pure solvent, analyzed to assess baseline stability.

Initial Calibration Verification (ICV): A standard prepared from a secondary source analyzed to verify the calibration standards.

Laboratory Control Sample (LCS) (also referred to as a Blank Spike): A sample of known concentration used as a basis for comparison with test samples that undergoes sample processing identical to that carried out for test samples.

Laboratory Duplicate Sample: One of two sample aliquots obtained from the same sample container and carried through the entire analytical process. Also referred to as a split sample.

LIMS: Laboratory Information Management System.

Matrix Spike: A second aliquot of sample spiked with a known amount of the analyte, used to assess matrix interference.

Method Blank: A blank sample that undergoes sample processing identical to that carried out for test samples. Method blank results are used to assess contamination from the laboratory environment and reagents.

Relative Percent Difference (RPD): The absolute difference between two results expressed as a percentage of the average result:

$$RPD = \left| \frac{(x_1 - x_2)}{(x_1 + x_2)/2} \right| \times 100$$

Standard Deviation (SD): A measure of the dispersion or imprecision of a sample or population distribution expressed as the positive square root of the variance, with the same unit of measurement as the mean.

Relative Standard Deviation (RSD): The standard deviation of an array *X* divided by the average of the array, times 100. Expressed as a percentage:

$$RSD = [SD_{(1\text{-}X)} / \text{average}_{(1-X)}] \times 100$$

4 Reference Method

4.1 Primary Reference

"Determination of Elemental Sulphur in Bottom Sediments Using High-Performance Liquid Chromatography", I. N. Azarova, *et al*., *Journal of Analytical Chemistry*, Vol. 56, No. 10, 2001, pp. 929–933.

4.2 Secondary Reference

"Measurement of Elemental Sulphur in Soil and Sediments: Field Sampling, Sample Storage, Pretreatment, Extraction and Analysis by High-performance Liquid Chromatography", J.H. Watkinson *et al*., *Aust. J. Soil Res*. 1987, 25, 167-178.

4.3 Deviations from the Primary Reference

Deviations have been demonstrated fit for purpose by method validation and performance.

Reference Method	SOP	Justification
5 g sample extracted with 3 x 15 mL portions of acetone in an ultrasonic bath	2 g sample extracted with 20 mL acetone, sonicated for 30 min. followed by 1 hr. tumbling	Excellent recoveries up to 400 mg/L
5 g sample extracted with 3 x 15 mL portions of acetone in an ultrasonic bath	2 g samples also extracted with 20 mL DCM	S8 has 10x greater solubility in DCM than in acetone. Allows determination of up to 6% S8 in soil
Acetonitrile-water mobile phase	Methanol-water mobile phase	S8 has greater solubility in methanol allowing a wider linear range than the reference method
Column (2 x 75 mm) packed with the sorbent Nucleosil 100-5 C18	AkzoNobel Kromasil C18 column (4.6 x 100 mm, 3.5 µm particle size)	Commercially available, higher capacity, provides excellent separation of the S8 peak

5 Regulatory Criteria

500 mg elemental S/kg of soil per Alberta Tier 1 Soil and Water Remediation Guidelines, May 23, 2014, or as amended.

6 Safety and Disposal

6.1 The use of personal protective equipment, including safety glasses and lab coats is required. It is the responsibility of the analyst to ensure that any additional identified hazard controls are used (e.g. nitrile gloves, splash goggles, fume hoods, respirators, etc.)

6.2 Material safety data sheets for all chemical reagents must be available to personnel using this method. Staff performing this method shall review the associated MSDS sheets for chemicals used in this procedure and ensure they understand the associated hazards and safety controls required to work safely with each chemical.

6.3 Dispose of all samples, extracts and reagents according to local, provincial and federal laws and regulations.

6.4 CAUTION: Samples containing > 30% S8 may spontaneously ignite on grinding. Suspected high S8 samples should be tested using minimal sample aliquots and not ground if ignitability is suspected.

7 Sample Handling, Preservation and Hold Time

Matrix	Container	Minimum Amount	Hold Time	Preservation
Soil Extract	Conical glass tubes with caps	10 mL	14 days	Store at 4 ± 2°C
Soil	Wide mouth glass jars (recommended*), plastic bags or polyethylene containers of various sizes.	125 mL	14 days**	Store at 4 ± 2°C.
Dried and Ground Soil	Glass	10 grams	indefinite	Store at room temperature

* S8 may tend to adhere electrostatically to plastic surfaces

** Soils should be dried and ground as soon as possible after receipt in order to minimize biological degradation of S8. Freezing as-received samples may be used to extend hold time.

8 Interferences

8.1 Acetone extracts with S8 concentrations ≥ 400 mg/L and DCM extracts with concentrations ≥ 6,500 mg/L must be re-extracted at a higher solvent:soil ratio. Studies have shown that soils with concentrations that yield extract concentrations above these levels are not completely extracted.

8.2 Co-extracted organics could potentially interfere with the S8 peak. HPLC conditions have been optimized such that the S8 peak elutes after most of these interferences.

8.3 S8 standards contain small amounts of S6 and S7 allotropes. Our studies have shown that these and other impurities are < 2% of the S8, therefore not significant.

8.4 Leaf litter samples may contain pine pitch or other interferences that can clog the guard column relatively quickly. Frequent (daily) guard column replacement will be required if high numbers of such samples are run.

9 Detection Limit

9.1 Method Detection Limit (MDL)

MDL must be re-determined at a minimum every two years or if a major change is introduced to the test method. Typical MDLs are 7 mg/kg for an acetone extract and 35 mg/kg for a DCM extract.

9.2 Reporting Limit (RL)

Acetone extract RL = 10 mg/kg. DCM extract RL = 40 mg/kg. Data less than the RL should be reported as < (RL) on the Certificate of Analysis. Any applicable sample dilution factors are applied to the RL values, which are reported accordingly.

9.3 Measurement of Uncertainty

The uncertainty value represents the variability of the result due to both random and systematic causes, thus providing a level of confidence when making a decision using a result. Bias estimates less than 5% are not included in this procedure.

9.4 Accuracy and Precision

The accuracy and precision of the method were estimated from replicate analyses of spiked real sample matrices. Accuracy is calculated based on the overall averaged recovery, and precision is assessed using relative standard deviation.

10 Glassware Cleaning

- Clean all glassware according to applicable glassware cleaning work instructions.
- Check for and dispose of damaged or stained glassware.
- Do not oven-dry volumetric glassware.

11 Apparatus and Materials

Where a brand or model is specified, an equivalent may be substituted.

11.1 Materials

- Analytical balance (readable to 0.1 mg) (Mettler or equivalent)
- End-over-end tumbler apparatus (Rotomixer or equivalent)
- Variable volume pipettors (Eppendorf or equivalent) (0.100 – 5 mL capacity)
- Centrifuge
- Spatula, metal
- 50 mL graduated conical tubes with caps
- Sonicator
- Class A volumetric pipettes 1 mL, 5 mL, 10 mL, 20 mL, 25 mL
- Dispenser capable of dispensing up to 50 mL
- Class A graduated cylinder
- 0.45 µm syringe filter
- Forced Air Oven, monitored with NIST traceable expanded range thermometer
- 2 mm sieve (10 mesh)
- Mortar and pestle or polymix grinding mill
- Plastic test tube rack

11.2 Apparatus

- Dionex LC System Specification
  - HPLC Pump: P580 A HPG, binary gradient pump, programmable up to 500 bar
  - 1 µl to 10 mL/minute floating rate, for micro and regular HPLC
  - ASI-100 Automated Sample Injector: in-line injection, carousel for 4 mL vials
  - Thermostated column compartment
  - 170S UVD Diode array UV/Vis detector
  - UCI 100 universal chromatography interface
  - Chromelon software, ver. 6.8 SR7
- AkzoNobel Kromasil C18 column (100 mm x 4.6 mm, 3.5 µm particle size)
- Guard Column, C18 (10 mm x 4.6 mm, 5 µm particle size)

12 Reagents and Standard Preparation

All chemicals used for the preparation of reagents and standards must be ACS grade or better unless otherwise stated. All solids must be weighed using calibrated and verified balances. Liquids must be measured using class A volumetric glassware or verified dispensers. Pipettors must be verified daily or before use.

12.1 Reagents

- **Reagent Water:** Deionized water (DI). Check conductivity daily. Acceptance limits are set at Type II water, < 1 µS/cm @ 25°C.
- **Acetone:** Distilled in glass or equivalent grade. Store as received at room temperature. The default expiry date is as specified on the manufacturer's label, or on the Certificate of Analysis provided by the manufacturer. If no expiry date is provided, the default expiry date is five (5) years from date of receipt or until deterioration is noted - whichever is earlier.
- **Dichloromethane:** Distilled in glass or equivalent grade. Store as received at room temperature. The default expiry date is as specified on the manufacturer's label, or on the Certificate of Analysis provided by the manufacturer. If no expiry date is provided, the default expiry date is five (5) years from date of receipt or until deterioration is noted - whichever is earlier.
- **Methanol:** HPLC Grade or equivalent. Store as received at room temperature. The default expiry date is as specified on the manufacturer's label, or on the Certificate of Analysis provided by the manufacturer. If no expiry date is provided, the default expiry date is five (5) years from date of receipt or until deterioration is noted - whichever is earlier.
- **Ottawa Sand:** Commercially purchased (Fisher Scientific Cat # S23 or equivalent). Store in a glass jar at room temperature. The default expiry date is as specified on the manufacturer's label, or on the Certificate of Analysis provided by the manufacturer. If no expiry date is provided, the default expiry date is five (5) years from date of receipt or until deterioration is noted - whichever is earlier.
- **Elemental Sulphur (S8):** Sulphur, precipitated, U.S.P, >99% purity, Fisher Chemical, Catalog N S595, Sulfur Sigma- Aldrich 13803-1KG-R 99.5-100.5%, or equivalent. Store as received at room temperature. The default expiry date is as specified on the manufacturer's label, or on the Certificate of Analysis provided by the manufacturer. If no expiry date is provided, the default expiry date is five (5) years from date of receipt or until deterioration is noted - whichever is earlier.

12.2 Calibration Standards

12.2.1 Stock Calibration Standard (400 mg/L S8): Dissolve 0.200 ± 0.002 g sulphur in DCM solvent and sonicate for at least 30 min until dissolved, then dilute to the volume in a 500 mL class A volumetric flask. Immediately transfer to a 500 mL amber glass bottle with Teflon®-lined lid, store at room temperature. The expiry date is 6 months from the date of preparation.

12.2.2 Working Standards: Into a series of 50 mL class A volumetric flasks, pipette suitable aliquots (see table below) of the Stock Calibration Standard, bulk to volume with acetone. Store in tightly capped glass vials at 4 ± 2°C. Stable for seven days. Acetone is highly volatile; keep vials tightly capped to prevent evaporation.

Standard	Volume of Stock Solution (mL)	Final Volume (mL)	Concentration (S8, mg/L)
1	0	50	0
2	0.125	50	1
3	0.25	50	2
4	1.25	50	10
5	6.25	50	50
6	12.5	50	100
7	25.0	50	200
8	50.0	50	400

12.3 Quality Control Standards

Quality control (QC) standards must be prepared from a secondary source.

12.3.1 Stock QC Standard Low (400 mg/L S8): Dissolve 0.200 ± 0.002 g S8 in acetone and sonicate for at least 30 min until dissolved then dilute to the volume in a 500 mL class A volumetric flask. Immediately transfer the solution into a 500 mL amber glass bottle with Teflon®-lined lid. Store at room temperature. The expiry date is 6 months from the date of preparation.

12.3.2 Stock QC Standard High (4,000 mg/L S8): Dissolve 2.00 ± 0.02 g S8 in DCM and sonicate for at least 30 min until dissolved, then dilute to the volume in a 500 mL class A volumetric flask. Immediately transfer the solution into a 500 mL amber glass bottle with Teflon®-lined lid. Store at room temperature. The expiry date is 6 months from the date of preparation.

12.3.3 ICV (100 mg/L S8): Add 25 mL of Stock QC Standard Low (400 mg/L S8) to a 100 mL class A volumetric flask. Fill to volume with acetone. Store in tightly capped glass vials at 4 ± 2°C. Stable for seven days. Acetone is highly volatile; keep vials tightly capped to prevent evaporation.

13 Sample Preparation

Note: Depending on the nature of the samples to be analyzed, deviations from the following protocol may be required. Any non-conformances must be documented.

1. Dry soils samples at 55 ± 5°C, grind and pass through a 2 mm sieve to ensure sample homogeneity. If the dried and ground soil sample contains visible sulphur, manually homogenize it with a pestle in a mortar. Weigh 2.00 to 2.10 g of dried and ground soil samples into 50 mL conical glass tubes. Record exact weight taken.

   CAUTION: Samples containing > 30% S8 may spontaneously ignite on grinding. Suspected high S8 samples should be tested using minimal sample aliquots and not ground if ignitability is suspected.

2. Add 20.0 ± 0.4 mL of acetone or DCM (depending on whether the high or low level method is required) using a verified dispenser.
3. Arrange tubes in a plastic test tube rack and sonicate for 30 ± 5 minutes.
4. Remove tubes from tray and remove excess water from the outside of the tubes. Rotary extract soil samples on an end-over-end tumbler apparatus (Rotomixer) for at least 1 hour at the highest setting.
5. Centrifuge at 2,000 rpm for at least 2 minutes or as long as necessary to separate the soil and solvent layers.
6. If sample extract appears turbid, filter using a 0.45 µm syringe filter.
7. Analyze acetone extracts undiluted. Dilute DCM extracts 10× with acetone prior to analysis.
8. For each batch of samples, the following quality control samples must be prepared and analyzed:
   a) Method Blank: 2.0 ± 0.1 g of Ottawa sand is used for the method blank.
   b) Sample Duplicate: Prepare a duplicate by processing a second subsample through the procedure.

c) Laboratory Control Sample (1,000 mg/kg): Spike 2.0 ± 0.1 g of Ottawa sand with 5 mL of the QC Stock Standard Low (Section 12.3.1). Add an additional 15 mL of acetone or DCM, as applicable, to bulk up to 20 mL total volume for the extraction and carry through the extraction and analysis procedure. For the high level method use QC Stock Standard High, (Section 12.3.2) (10,000 mg/kg).

d) Matrix Spike (1,000 mg/kg): Obtain a second subsample and add 5 mL of the QC Stock Standard Low (Section 12.3.1) directly onto the sample. Add an additional 15 mL of acetone or DCM, as applicable, to bulk up to 20 mL total volume for the extraction and carry through the extraction and analysis procedure. For the high level method use QC Stock Standard High, (Section 12.3.2) (10,000 mg/kg).

14 Analytical Determination

14.1 Instrument Setup

The following are typical instrument settings (these can be adjusted as required, to ensure optimum instrument performance):

Typical Instrument settings for Elemental Sulphur

- Column: C18, 100 mm x 4.6 mm ID, 3.5 µm particle size, or equivalent
- Guard Column: C18, 10 mm x 4.6 mm ID, 5 µm particle size, or equivalent (recommended for leaf litter soils)
- Column Flow: 2.3 mL/min
- Mobile Phase: Methanol/Water =90/10 (v/v)
- Wavelength: 220 nm
- Column Temperature: 45°C
- Injection Volume: 10 µL

14.2 Column Performance Check

S8 must be present at normal response. Check the calibration standards using the feature "peak analysis" for retention time, resolution and asymmetry. The minimum allowable retention factor, *k'* of the S8 peak should be ≥ 7.0 to allow adequate separation of the S8 from interferences.

14.3 Instrument Calibration

14.3.1 Initial Calibration

A multi-point calibration is to be run at the concentrations indicated in Section 12.2.2, with the lowest concentration analyzed first and the highest concentration analyzed last.

The calibration curve is linear and can be based on Response Factor (RF), linear regression with an intercept, or linear regression forced through zero. Method validation showed that linear regression with 1/x weighting provides more accurate data at concentrations near the reporting limit.

$$y = mx + b$$

$$x = (y - b)/m$$

Where:

x = Concentration of the analyte

y = the peak area of the analyte

m = the slope of the calibration curve

b = the y intercept of the curve.

14.3.2 Calibration Acceptance Criteria

The correlation coefficient (r) should be ≥ 0.995.

The residuals of the standards (the concentration of each standard as calculated from the curve), should be:

Standard	Concentration (S8 mg/L)	Residual
1	0	± 1
2	1	± 50%
3	2	± 25 %
4	10	± 10%
5	50	± 10%
6	100	± 10%
7	200	± 10%
8	400	± 10%

Calibration using Response Factors, RF, is an acceptable alternative and is essentially the same as y = mx except all points have equal weight. The response factor for each of the analytes can be calculated as follows:

$$RF = \frac{A_S}{C_S}$$

Where:

A_S = Peak area of the analyte

C_S = Concentration of the analyte

The %RSD (relative standard deviation) of all the individual RFs should be ≤ 20%.

%RSD = 100*$SD_{\Sigma RF}$/average RF where $SD_{\Sigma RF}$ = the standard deviation of the individual RFs

If %RSD ≤ 20%, the average RF may be used for quantitation of sample data.

14.3.3 Initial Calibration Verification (ICV) and Continuing Calibration Verification (CCV)

The initial calibration verification standard (secondary standard at 20 mg/L concentration) is run to verify the initial calibration curve. The concentration of S8 present in the ICV should fall within80% to 120% of the expected value.

Continuing calibration verification (CCV) standards are prepared from the primary reference standard and should bracket each batch of samples. A CCV will be analyzed every 20 samples and end of each batch. All parameters must be within 80% to 120% of the expected value. If considered necessary, CCVs can be run more frequently.

The percent drift, or deviation from calibration curve, can be calculated using the following formula:

$$\%DRIFT = \frac{|CI - CC|}{CI} * 100$$

where:

CI =Design concentration of the CCV standard,

CC = Calculated concentration of the CCV standard using the initial calibration curve.

If the CCV doesn't meet the above criterion, the instrument may require maintenance and/or full calibration.

The retention time of the standard in the CCV must be evaluated: if the retention time of standard changes by more than 30 seconds from that in the mid-point standard level (of the most recent initial calibration sequence) the chromatographic system must be inspected. When

corrections are made, the samples analyzed while the system was malfunctioning must be re-analyzed.

14.3.4 Run Format

Analyze the standards and QC standard as per the run queue specified below. The sequence below is an example. The actual run sequence may vary somewhat provided all calibration and QC elements are maintained at the required frequency.

Sample Name	Type
0 mg/L	Calibration Standard
1 mg/L	Calibration Standard
2 mg/L	Calibration Standard
10 mg/L	Calibration Standard
50 mg/L	Calibration Standard
100 mg/L	Calibration Standard
200 mg/L	Calibration Standard
400 mg/L	Calibration Standard
ICB (0 mg/L)	QC
ICV (100 mg/L)	QC
Method Blank	QC
Laboratory Control Sample	QC
Samples 1-20	Samples
Laboratory Duplicate Sample	QC
Matrix Spike	QC
Laboratory control sample	QC
CCB (Standard 1, 0 mg/L)	QC
CCV (Standard 6, 100 mg/L)	QC

15 Quality Control Requirements

QC Requirement	Frequency	Acceptance Limits	Corrective Action for Data Outside Limits
Calibration	Minimum Monthly	Linearity (Correlation Coefficient) is ≥ 0.995 + residuals as above	1. Re-analyze original calibration standards. 2. Re-prepare and reanalyze calibration standards.
CCV	1 / batch of 20 or fewer samples	80 – 120% Recovery	1. Re-analyze the standard. 2. Re-prepare new standard 3. Re-calibrate the instrument 4. Do not report data without bracketing QC.
ICB and CCB	1 / batch of 20 or fewer samples	Abs(CCB, ICB) <RL	1. If positive blanks are detected at levels ≥ 2xRL, repeat the analysis of the ICB or CCB. 2. If ICB or CCB still fails, recalibrate and re-analyze the batch.
ICV	1 / batch of 20 or fewer samples	80 - 120% Recovery	1. Re-analyze the external reference. 2. Re-prepare new standard.
Method Blank	1 / batch of 20 or fewer samples	Absolute (MB) < (RL)	1. If positive blanks are detected at levels ≥ RL, repeat the analysis of the method blank. 2. If the blank still fails, re-prepare and re-analyze the batch.
Laboratory Control Sample	1 / batch of 20 or fewer samples	80 – 120%	1. Reanalyze the laboratory control sample. If it fails again find and correct the problem. Corrective action may include repeating the batch. 2. The laboratory control sample may not be reported outside ±3SD unless circumstances do not permit reanalysis (e.g. hold time or limited sample volume issues).

QC Requirement	Frequency	Acceptance Limits	Corrective Action for Data Outside Limits
Laboratory Duplicate Sample	1 / batch of 20 or fewer samples	± 30% RPD[1]	1. Re-analyze the sample and duplicate. 2. Re-prepare new sample and duplicate.
Matrix Spike	1 / batch of 20 or fewer samples	70 – 130% Recovery[2]	1. Re-prepare and repeat the analysis of the matrix spike. 2. If failure is due to matrix effect, report sample with a comment in LIMS and fill out a bench level non-conformance report.
Method Blank	1 / batch of 20 or fewer samples	Absolute (MB) < (RL)	1. If positive blanks are detected at levels ≥ RL, repeat the analysis of the method blank. 2. If the blank still fails, re-prepare and re-analyze the batch.

[1]These criteria are not applicable for duplicates that are less than 5 X RL.
[2]Matrix spike is non-calculable when the sample concentration is greater than the spiked concentration.

Examine the need to reanalyze or re-extract the entire batch of samples if the corrective actions in the table are not effective, and it is believed the issue(s) may affect the entire batch. If the QC failures cannot be localized to the specific QC sample then it can be inferred that there is an instrumental problem, which must be resolved prior to sample analysis.

16 Data Interpretation, Calculations and Data Reporting

16.1 Calculations

The concentrations of the calibration standards are plotted on the x-axis of the calibration curve, with the peak area of the calibration standards plotted on the y-axis. The calibration curve is determined from linear regression with 1/x weighting. The equation of the trend line is in the format y = mx + b.

The concentration of samples is calculated from the calibration curve from the following equation:

$$C_x = (A_x - b)/m$$

where:

C_x = concentration of sample in the extract as injected (mg/L)

A_x = peak area of sample

m = slope of the calibration curve

b = y intercept of the calibration curve.

Use the following equation to convert the measured concentration of the injected solution to soil concentration:

$$C_s = C_x \times DF \times V_e/W_s$$

where:

C_s = concentration of S8 in the sample (mg/kg dry weight),

DF = dilution factor of the extract, normally 1 for acetone and 10 for DCM,

V_e = volume of extract in mL, normally 20 mL,

W_s = weight of dried, ground sample in g, normally 2 g.

16.2 Analyte Identification

The retention time for the sample should be within 0.2 minutes of the retention time of the most recent CCV or ICV.

16.3 Significant Figures

Carry at least three significant figures through all calculations. Results are normally reported to two significant figures unless otherwise requested by the data user.

17 Column Maintenance

17.1 Column Cleaning Protocol

Kromasil C18, 100 mm length, 4.6 mm diameter, 3.5 µm particle size

If highly retained components cannot be eluted off the column, the column has lost its capacity and is considered contaminated. Contaminants can come from the eluent and/or the sample. Depending upon the contaminant, the column should be cleaned or replaced. To protect column from contamination use:

- Pre-column filter - Supelco Column Saver 0.5 µm Filter, Catalogue No. 55214U (or equivalent)
- Supelco Guard Cartridge for 3.2-4.6mm ID columns, Catalogue No. Z227137, installed in Guard Cartridge Holder, Catalogue No. 54987 (or equivalent)

If system back pressure increases by 5%, replace Pre-column filter and Guard Cartridge.

During routine use, the columns may show signs of deterioration. The effects of column deterioration can be seen as any of the following:

- Loss of retention
- Loss in resolution
- Abnormal peak shapes
- Abnormal operating pressures

The following cleaning procedures should regenerate the performance of the column:

- Remove the guard column and connect Kromasil 100-3.5C18 analytical column to the pump
- Flush the column with Acetonitrile, HPLC grade, 99.9% purity, for 1-1.5 hour at flow rate 3 mL/min
- Flush the column with Methanol, HPLC grade, 99.9% purity for 15 minutes at flow rate 2.3 mL/min
- Equilibrate the column with Mobile Phase (90:10 Methanol:Water) for 30 minutes at flow rate 2.3 mL/min until a stable baseline is achieved.

If the column performance is still poor, replace the analytical column.

Attachment B. Elemental Sulphur Methods Round Robin

Development and Validation of Analytical Methods for Elemental Sulphur in Alberta Soils

Prepared by Maxxam Analytics for Alberta Environment and Parks

June, 2015

This round robin project was coordinated by Maxxam Analytics (MAXXAM) to evaluate the performance of two new HPLC methods that MAXXAM recently developed for the Government of Alberta. The methods were compared with alternative methods with a range of testing materials.

1 Study Design

Duplicate samples were provided with weights as requested by the participants (Table 1). All samples were dried at 55 – 60°C, ground and sieved through a 2 mm sieve. Spiked samples were prepared by weighing individual aliquots into tubes and spiking with the appropriate amount of a concentrated elemental sulphur spiking solution prepared in acetone or DCM depending on concentration. The solvent was allowed to evaporate overnight in a fumehood. The tube contents were mixed and the tubes capped, labeled and distributed. Participants were instructed to extract the entire sample, preferably in the tube, but if not, to rinse the tube with solvent.

Table 1: Requested Sample Weights, Lodgepole Pine, High Organic Clay, Peat

Method	Sample Weight
HPLC Ref	2 g
Colour 1	2 g
HPLC 2	1 g
Colour 2	1 g
ICP 1	5 g
ICP 2	5 g

The samples and design concentrations are summarized in Table 2.

The sample types were chosen to include the most challenging matrices found in Alberta soils.

- Leaf Litter is a common matrix for monitoring the impact from deposition of sulphur dust on soils. The extracts contain substantial levels of potential interferences for the HPLC and colourimetric procedures.
- High Organic Clay and Peat samples are traditionally the most difficult matrices from which to extract.
- The ESRD sample is a leaf litter sample containing elemental sulphur resulting from aerial emissions.
- The CRM was the only reference material we could find with a value for elemental sulphur. The value is informational, meaning that there was insufficient data and /

or insufficient number of independent analytical techniques to provide a certified value.

Table 2: Samples and Design Concentrations

Matrix	Design Concentration of Elemental S (µg/g)	Comments
Lodgepole Pine (LPP)	30	Leaf litter sample. Extracts highly coloured
Lodgepole Pine (LPP)	500	
Lodgepole Pine (LPP)	Unspiked	
High Organic Clay (HOC)	80	40% clay 9% TOC
High Organic Clay (HOC)	2000	
Peat	Unspiked	
Peat	30	
Peat	300	
ESRD Sample	Unspiked	Leaf Litter # 39, AS LP
CRM	5000 ± 1600	Ground rock, External Reference Material Natural Resources Canada, RTS-1. Elemental S concentration value is informational, not certified.

2 Analytical Methods

Six sets of data were generated by four participating laboratories

HPLC Ref: This is the method developed for the Department by Maxxam. Low level samples were extracted with acetone, high level samples with DCM. Extracts were analyzed by HPLC with UV detection at 220 nm using a C18 column, eluent 10% water in methanol, 10 µL injection volume.

HPLC 2: Samples were extracted with acetone and extracts analyzed by HPLC with UV detection at 255 nm using a C18 column, eluent 5% water in methanol, 2-40 µL injection volume.

Colour 1: Samples were extracted with acetone, a portion of the extract reacted with cyanide to form thiocyanate (NCS) and subsequently with ferric chloride ($FeCl_3$) to form a coloured ferrothiocyanate complex, measured at 465 nm. To correct for background, the absorbance of a sample without the $FeCl_3$ colour-forming reagent was measured, subtracted from the analytical sample, and the net absorbance used to calculate concentration.

Colour 2: The chemistry is the same as Colour 1 except no background correction was applied.

ICP 1 and ICP 2: Two different laboratories followed the same procedure. Samples were extracted with DCM (10:1 DCM:Soil ratio). The extract was evaporated to dryness and the residue acid digested prior to analysis for sulphur by ICPOES

3 Results

There were no outliers for any of samples as determined by the Grubbs test (P = 0.05). For labs that reported duplicate results, the duplicates were averaged prior to examination for outliers and data analysis. HPLC Ref and Colour 1 methods provided duplicate data. All duplicates >5x RL were <20% RPD, with one exception where the RPD for unspiked peat was 26%. HPLC Ref duplicates averaged 6% RPD (including the 26%) and Colour 1, 7% (Table 3).

3.1 Overall Trends

ICP 1 and 2 methods produced consistently lower results than other tested methods, and this bias was greater in the samples with higher organic carbon content (Appendix 1). This was unexpected. Further discussion with the labs suggested the bias was due to a solvent extraction time that was insufficient for complete recovery of elemental sulphur from the study samples. The ICP 1 and 2 methods used extraction times that were only ⅛ - ¼ the duration of the extraction time used for the HPLC reference method that was developed under this project. After the study, both the labs that produced the ICP results increased extraction times to 2 hours. The impact of this change could not be evaluated on the study samples because they had been exhausted. Prior to the study, there had been concern that the ICP method could possibly generate biased high results if organosulphur compounds were present in the samples. Further investigations are needed to address these issues.

The samples most likely to cause interferences on the colourimetric procedure were LPP, ESRD Sample # 39, and Peat. In every case, the Colour 2 method results were higher than Colour 1. This is in keeping with the fact that the Colour 1 method employs a correction for background colour whereas Colour 2 does not. Note also that the results for the unspiked lodgepole pine for Colour 1 (not background corrected) and Colour 2 methods were 47 and 124 mg/kg respectively whereas the ICP and HPLC results were all <RL, indicating a significant background absorbance for the colourimetric procedure.

HPLC 2 results were consistently lower than HPLC Ref. There was no obvious reason for this. Instrument and extraction conditions were similar for both methods; however, HPLC 2 used a different wavelength for detection and variable injection volumes.

3.2 Recovery of Spiked Samples

The recoveries of the spiked samples follow the same patterns discussed above.

Table 3. Average Recovery of Spiked Samples (excluding LPP30 and Peat 30*)

	Recovery	Stdev	Min	Max
HPLC Ref	107%	15%	97%	129%
Colour 1	100%	16%	123%	89%
HPLC 2	81%	6%	73%	90%
Colour 2	104%	21%	74%	117%
ICP 1	65%	11%	56%	80%
ICP 2	66%	22%	<33%	84%

*Because the level of S8 in the peat sample was unexpectedly high (262 mg/kg), the low level spike of 30 mg/kg was too low to provide meaningful recoveries and thus not included in the calculations. Similarly, the LPP 30 spike was <5x the RL for most participants and not included.

The ICP method recoveries were consistently low, the Colour 2 method recovery was high for all samples except the Peat-300 (Table 2). The HPLC 2 method also was low, but the most consistent (RSD 8%). The HPLC Ref and Colour 1 methods provided good overall recoveries. The Colour 2 method had recoveries > 100% for the LPP-500, HOC-80 and HOC-2000, balanced by a low recovery (74%) for the Peat-300 elemental S µg/g treatment resulting in the high RSD (21%). The HPLC Ref method had a 129% recovery for the Peat-300 spike and between 97% and 101% for the others. The peat sample was also very light which may have contributed to homogeneity issues as suggested by the high RSD for the duplicate of the unspiked sample.

CRM: As mentioned above, the reference value of 5000 ± 1600 mg/kg is informational. All labs reported values < 5000 mg/kg. The mean of all results was 3400 ± 660 mg/kg. After more data is collected, the information will be provided to the CRM provider.

A summary of all reported data is provided in Appendix B-1.

4 Conclusions

The HPLC method developed for the Government of Alberta performed well for a variety of different and difficult matrices, typical of Alberta forest soils. The colourimetric procedure,

when background correction is applied, also performed well. The low bias for the ICP procedure was unexpected and will require further investigation.

Data Analysis and Report
Preparation by

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Appendix B-1: All Reported Data

	CRM	ESRD	LPP	LPP	LPP	HOC	HOC	Peat	Peat	Peat
Design	5000		30	500	0	80	2000	30	300	0
HPLC Ref	3515	1423	33	478	<10	80	1965	262	621	252
	3464	1423	37	492	<10	80	2077	295	596	194
Colour 1	4524	1808	44**	465	47*	92	1864	300	543	290
			46**	473	50*	105		270	568	
HPLC2	2720	1360	27	425	<1	63	1460	226	460	199
Colour 2	3830	1920	69	585	124	93	2181	373	610	389
ICP 1	2970	1050	20	293	<10	45	1590	176	388	192
ICP 2	3100	1110	<10	177	<10	52	1680	233	512	277

mean	3439	1445	34	424	76	1831	267	537	256
stdev	661	356	21	129	21	265	59	81	71
RSD	19%	25%	62%	30%	28%	14%	22%	15%	28%

* uncorrected for background

** background corrected

Recovery	CRM	LPP 30	LPP500	HOC80	HOC2000	Peat30	Peat300
HPLC Ref	70%	117%	97%	100%	101%	185%	129%
Colour 1	90%	150%	94%	123%	93%	<	89%
HPLC2	54%	90%	85%	79%	73%	90%	87%
Colour 2	77%	230%	117%	116%	109%	<	74%
ICP 1	59%	65%	59%	56%	80%	<	65%
ICP 2	62%	<33%	35%	64%	84%	<	78%

Average Recovery of Spiked Samples (exc. LPP30 and Peat 30)				
	Recovery	**Stdev**	**Min**	**Max**
HPLC Ref	105%	15%	97%	129%
Colour 1	104%	16%	123%	89%
HPLC2	81%	6%	73%	90%
Colour 2	106%	21%	74%	117%
ICP 1	63%	11%	56%	80%
ICP 2	65%	22%	<33%	84%