Number: SHT-A2PREP-4
Date: November 11, 2013
Pages: 10

SERVICE How To

Model Number:	Originator:	Topic
A2PREP	**Petro van Poppel**	**System Checkout**

Full System Checkout

You will need:

- Standard Analytical Method (see SHT_A2PREP-2)
- Standard Preparative Method (see SHT_A2PREP-2)
- Solvent A: 99.9% water, 0.1% formic acid
- Solvent B: 99.9% ACN, 0.1% formic acid
- Make-up Solvent: 74% methanol, 25% ACN, 0.9% water, 0.1% formic acid
- Needle Purge Solution: 50% ACN
- Needle Wash Solution: 50%ACN
- Analytical Column: SB-C18 4.6x50mm, 5µm
- Preparative Column: SB-C18 21.2x50mm, 5µm
- System Check out Samples

Procedure:

1. Create a sequence, based on the Analytical method that is built as per SHT_A2PREP_2.

Line	Location	Sample Name	Method Name	Inj/Location	Inj Volume	Frac. Start	Target Masses
1	P1-C-01	testmix	ANALYT_BASIS_SHORT	1	10		194
2	P1-C-01	testmix	ANALYT_BASIS_SHORT	1	10	Next Location	166
3	P1-C-01	testmix	ANALYT_BASIS_SHORT	1	10	Next Location	180
4	P1-C-01	testmix	ANALYT_BASIS_SHORT	1	10	Next Location	228
5	P1-C-01	testmix	ANALYT_BASIS_SHORT	1	10	Next Location	100
6	P1-C-02	dye	ANALYT_BASIS_SHORT	1	10	Next Location	227

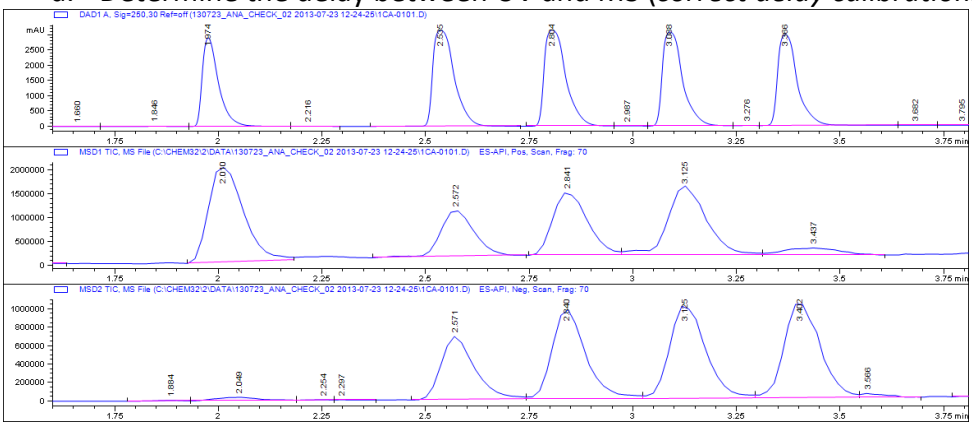
2. Run the sequence and place the Checkout solution (5 components) in position P1-C-01 and the dye solution (dark blue) in position P1-C-02.

3. 5 components of the spec out solution are:
   Caffeine (M=194); Methylparabene (M=152); Ethylparabene (M=166); Propylparabene (M=180); Benzylparabene (M=228).

4. In addition, dye solution - Thionine acetate (M=227).

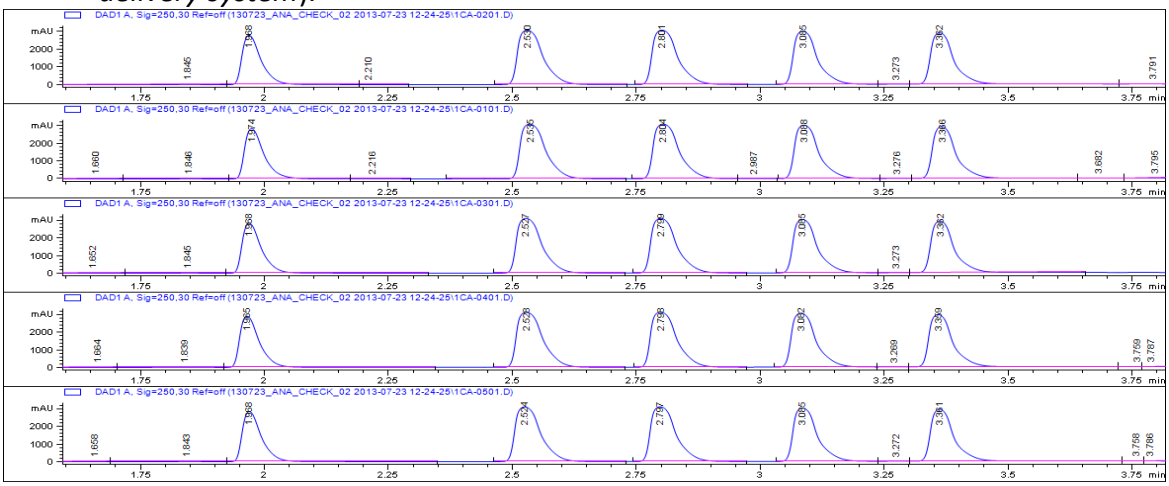
5. Evaluate the results of the analytical sequence, while looking at the following parameters:

    a. Determine the delay between UV and MS (*correct delay calibrations*).



Note: Make sure all plots line up with each other and that the delay calibration has corrected the delay differences.

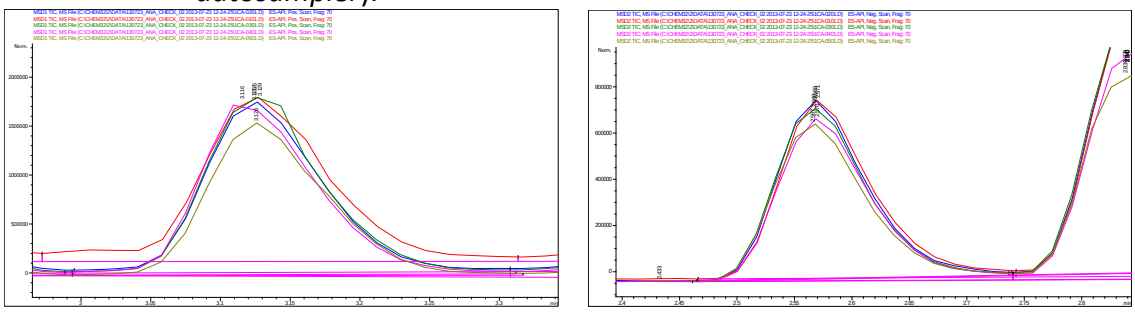
    b. Reproducibility of retention times (*correct functioning of the solvent delivery system*).



Note: Overlay the UV signals of the 5 injections. The retention time variation should be in a window of 0.1 minutes for caffeine (1st peak).

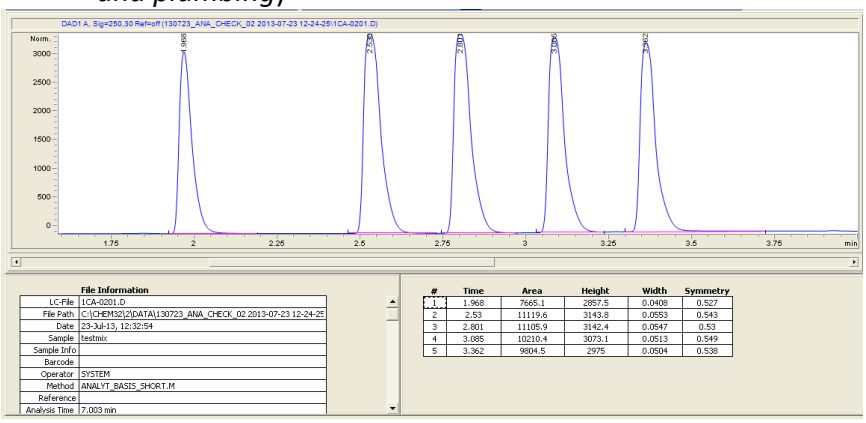
0.5% RSD is the specification for caffeine. Greater shifts in retention time than 0.1 minutes indicates technical issues with the solvent delivery system (the system is not purged properly, the system might have technical issues like leaks or not proper working check valves).

c. Reproducibility of peak height/peak area (*correct functioning of the autosampler*).



Note: The deviation of peak height should be better than 25%. Larger deviations than 25% in peak height indicates a problem with the dual loop ALS.

d. Measure peak width and symmetry (*correct functioning of the column and plumbing*)

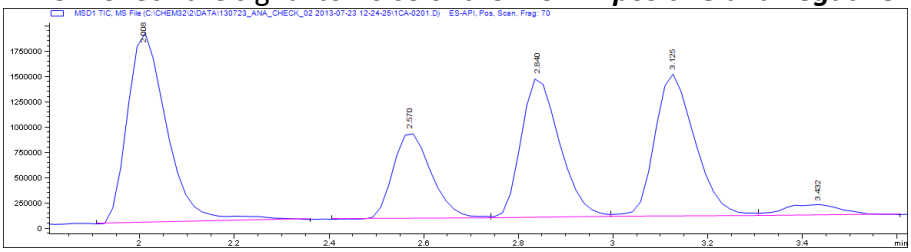


File Information	
LC-File	LCA-0201.D
File Path	C:\CHEM32\2\DATA\130723_ANA_CHECK_02 2013-07-23 12-24-25
Date	23-Jul-13, 12:32:54
Sample	testmix
Sample Info	
Barcode	
Operator	SYSTEM
Method	ANALYT_BASIS_SHORT.M
Reference	
Analysis Time	7.003 min

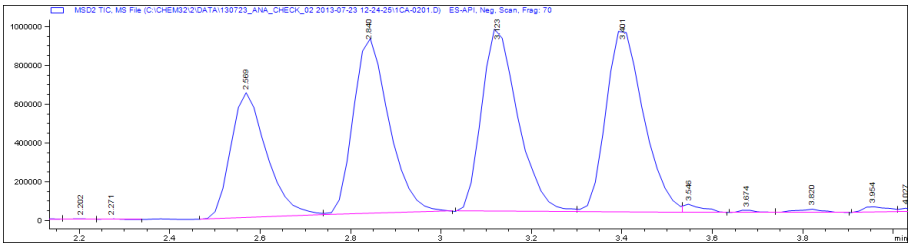
#	Time	Area	Height	Width	Symmetry
1	1.968	7665.1	2857.5	0.0408	0.527
2	2.53	11119.6	3143.8	0.0553	0.543
3	2.801	11105.9	3142.4	0.0547	0.53
4	3.085	10210.4	3073.1	0.0513	0.549
5	3.362	9804.5	2975	0.0504	0.538

Note: The peak width should be in the range of 0.1 minutes (half height). A strong fronting or tailing indicates problems with the column or dead volumes caused by improper plumbing. When the peak widths are too large there might be some problems with the column or with void volumes in the analytical flow path.

e. Check the signal to noise of the MSD in ***positive*** and ***negative*** TIC



Possitive mode

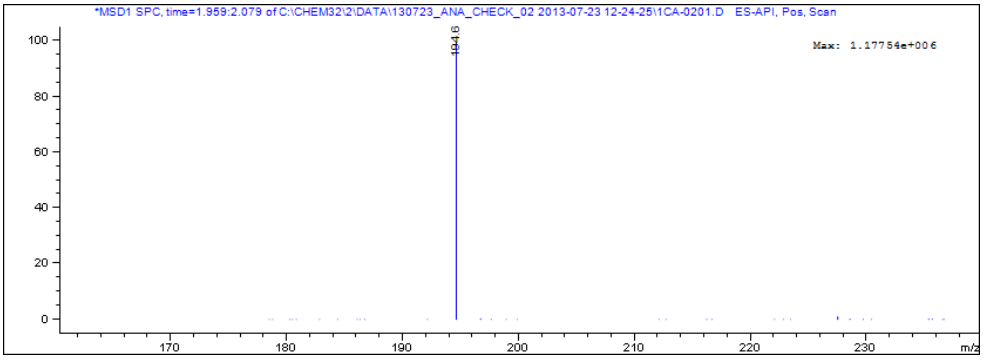


Negative mode

Note: The signal to noise ratio for the first peak (caffeine) should be better than 6:1, and for the second peak (methylparabene) it should be better than 3:1. If this is not the case, check if the flow into the ion source is correct.

The split ratio caused by the T-Splitter should be in the range of between 7.5:1 and 3:1 (200 – 500 µl in source). There could be some block of the capillary in the ESI probe.
A bad signal to noise ratio can be caused by contaminated solvents or bleeding columns. A further reason is a contaminated ion source which has to be cleaned.

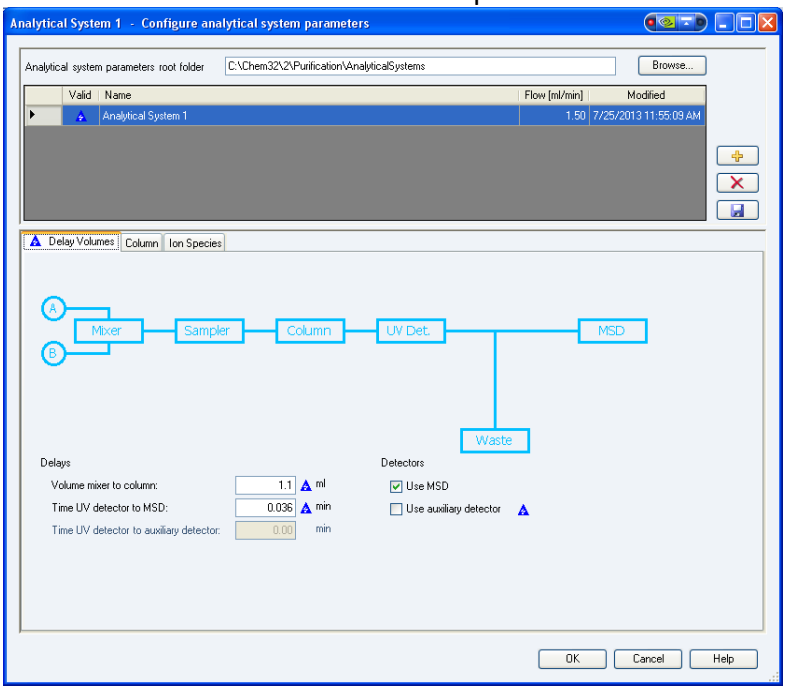
f. Check the measured mass values, do they match with the expected values?



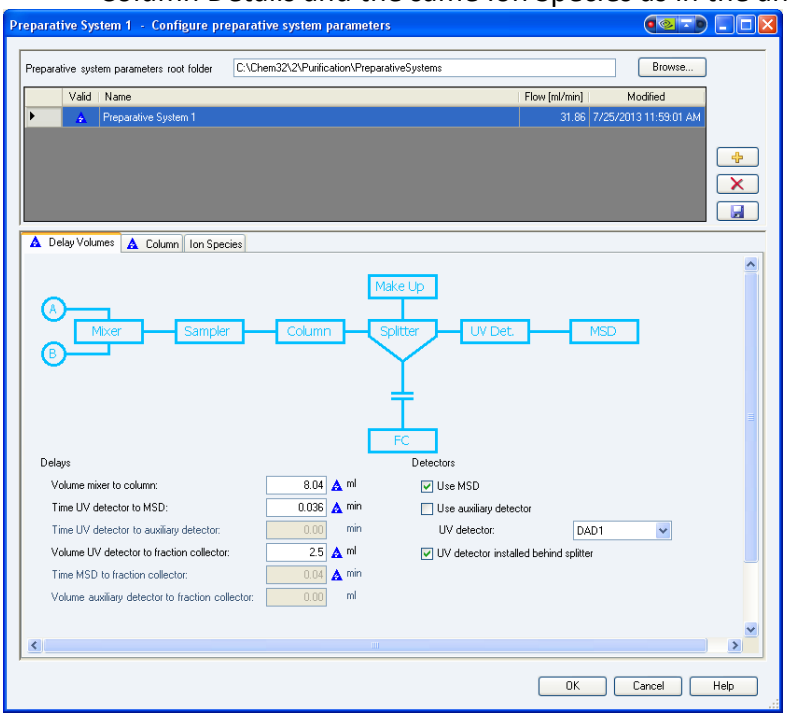
Note: The measured mass in this example is 194.6, where the expected mass is 194.1 AMU (+/-0.3Da). As this value is outside the tolerance, the mass spec has to be recalibrated (autotune function).

6. Set up the Purification Software:

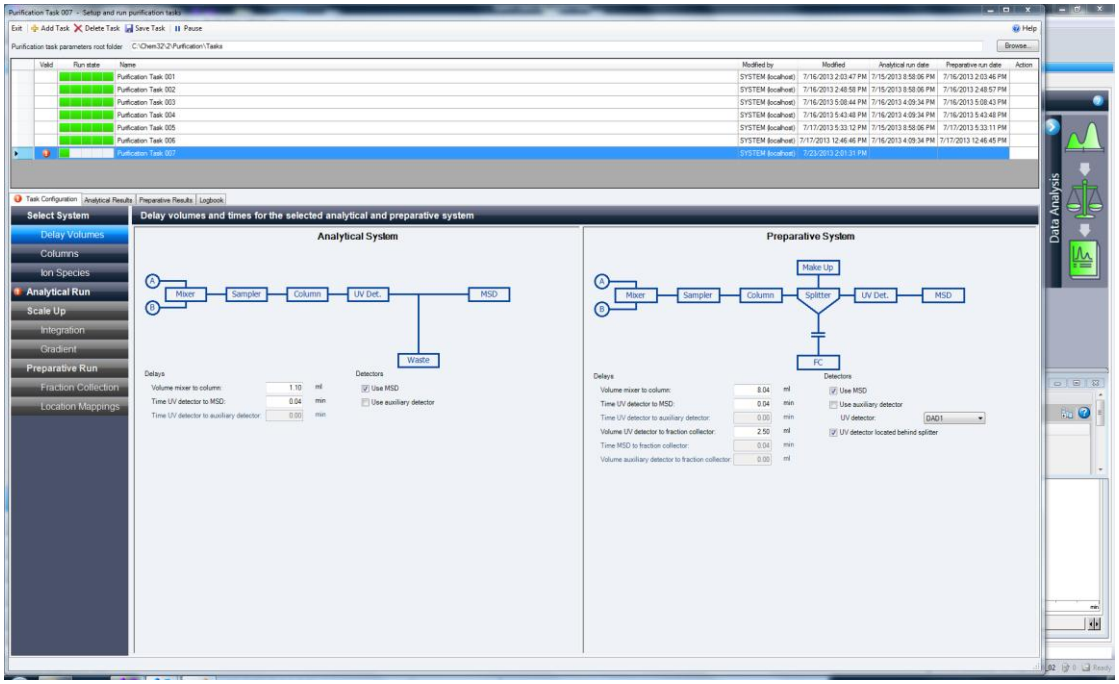
a. Define the analytical system, by specifying the Delay Volumes, the Column Details and the Ion Species.



b. Define the preparative system, by specifying the Delay Volumes, the Column Details and the same Ion Species as in the analytical system.

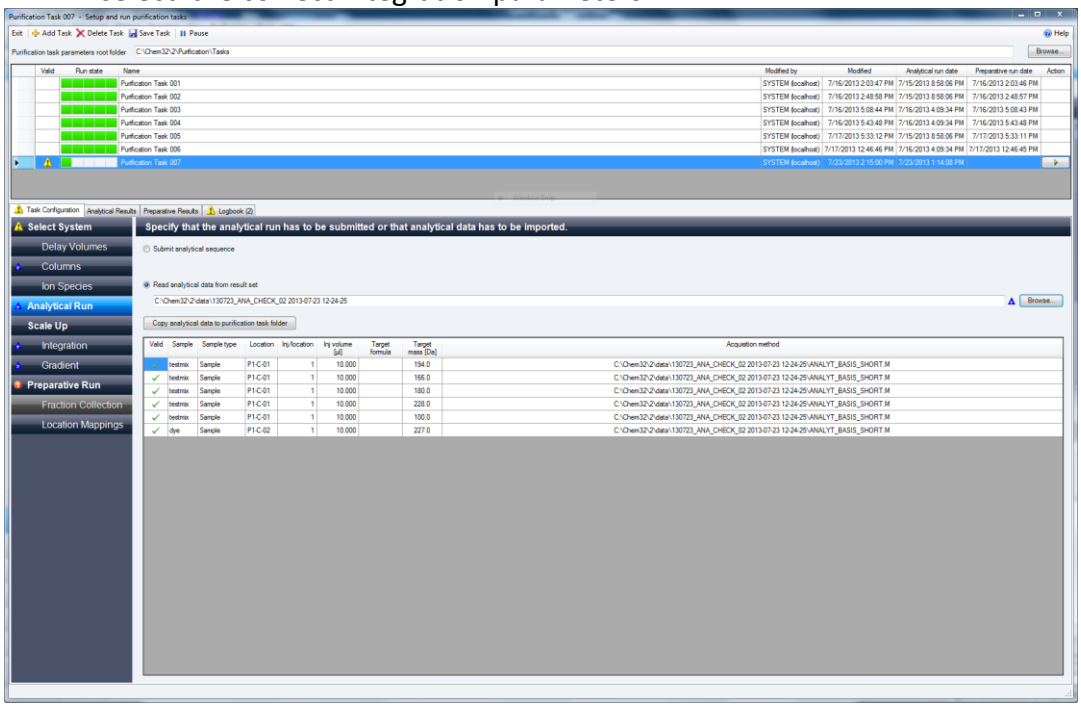


7. Create and start a task.

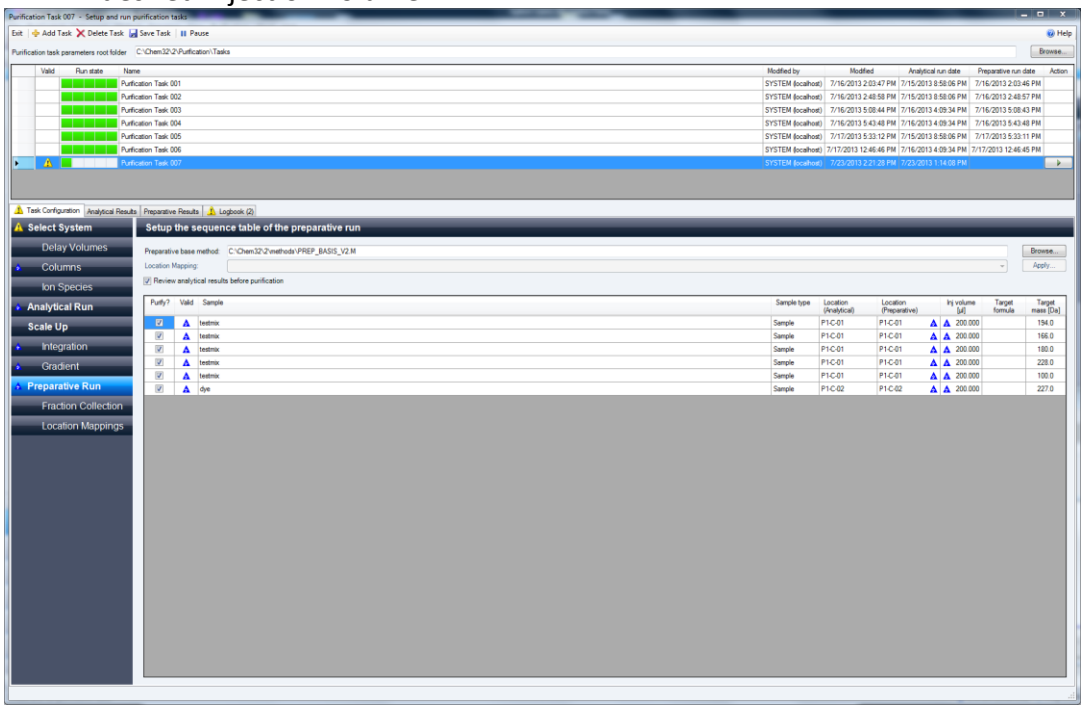


Check that all parameters are correct (selected column type, ions to monitor)

8. Load the result set of the analytical sequence and check if the different signals (UV and MS) are matching within the a determined window and select the correct integration parameters.



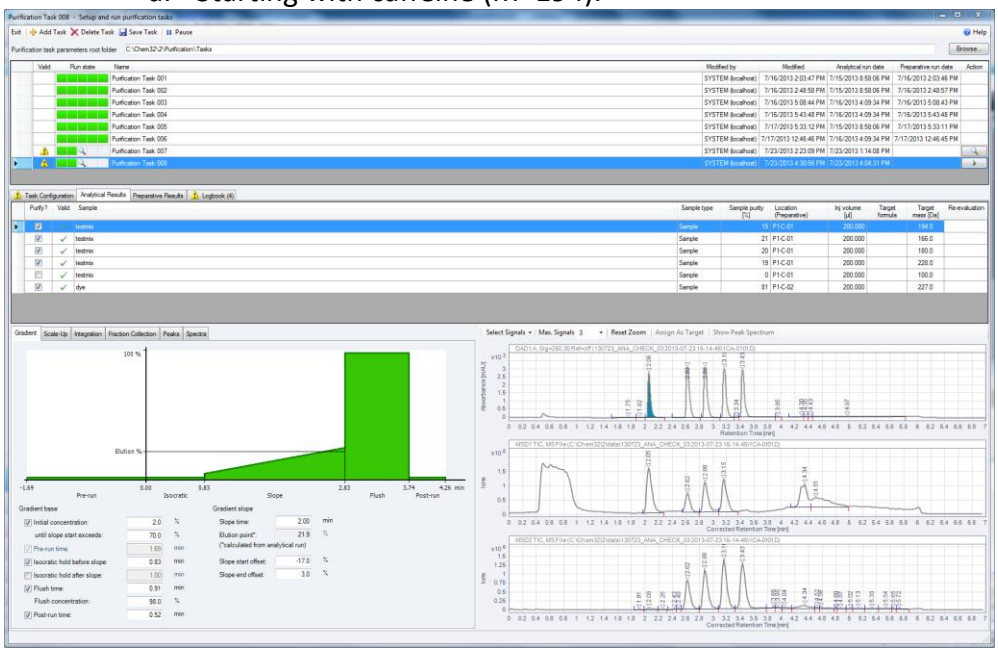
9. Select the preparative standard method the right sample location and the desired injection volume:



10. Double check the parameters of the fraction collector. The MSD will use threshold only, the UV detector will use threshold and slope for triggering. The time function for fraction collection should be aligned with the duration of the focused gradient.

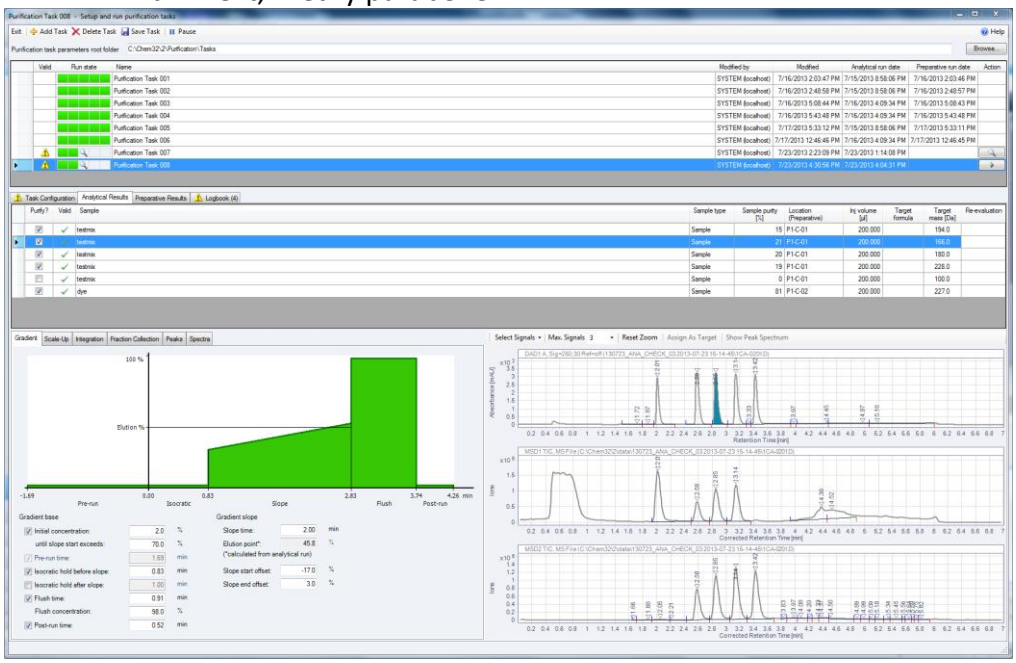
11. Hit the proceed button. The result set will be processed now.

12. After the sample has been processed just confirm the results:
    a. Starting with caffeine (M=194).



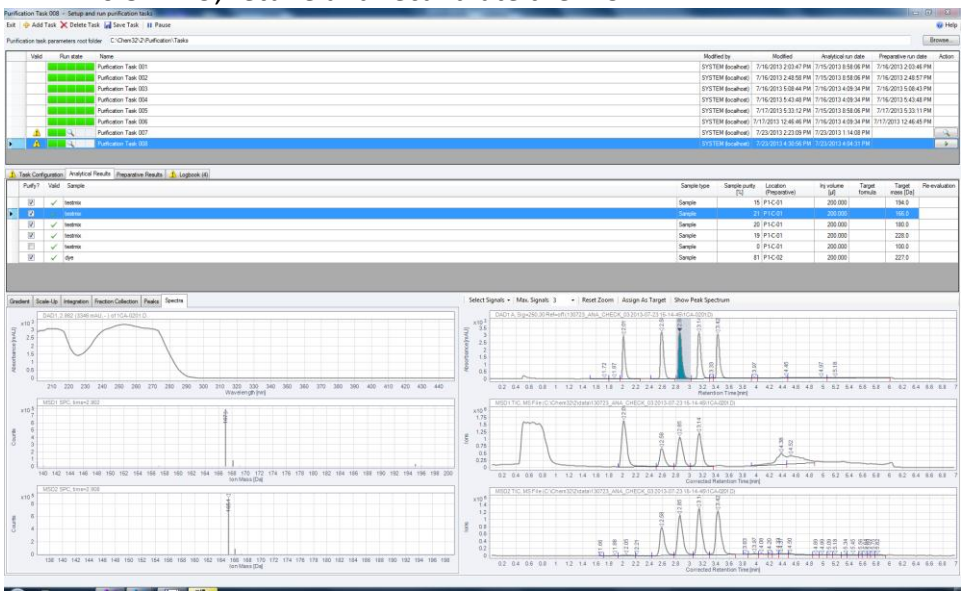
Note: Target No 1 (Caffeine, M=194) has been found, a focused gradient has been calculated.

    b. Next, Methylparabene.



Note: Target No 2 (Methylparabene, M=152) has been found, a focused gradient has been calculated.

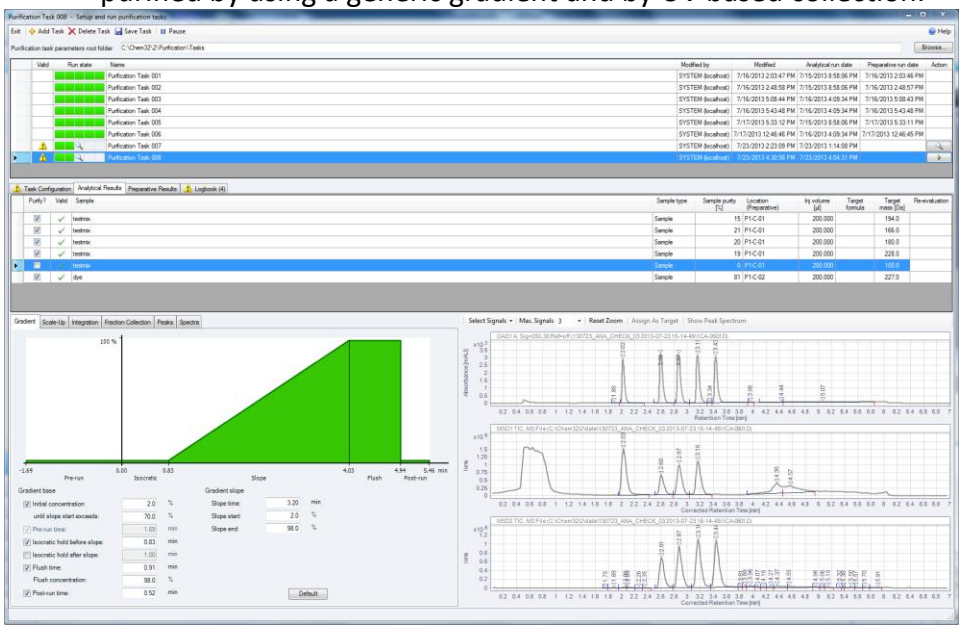
13. If the spectral data needs to be checked, select the spectra tab and click on the target peak. If the measured molecular weight difference is more than 0.3 AMU, retune and recalibrate the MSD.



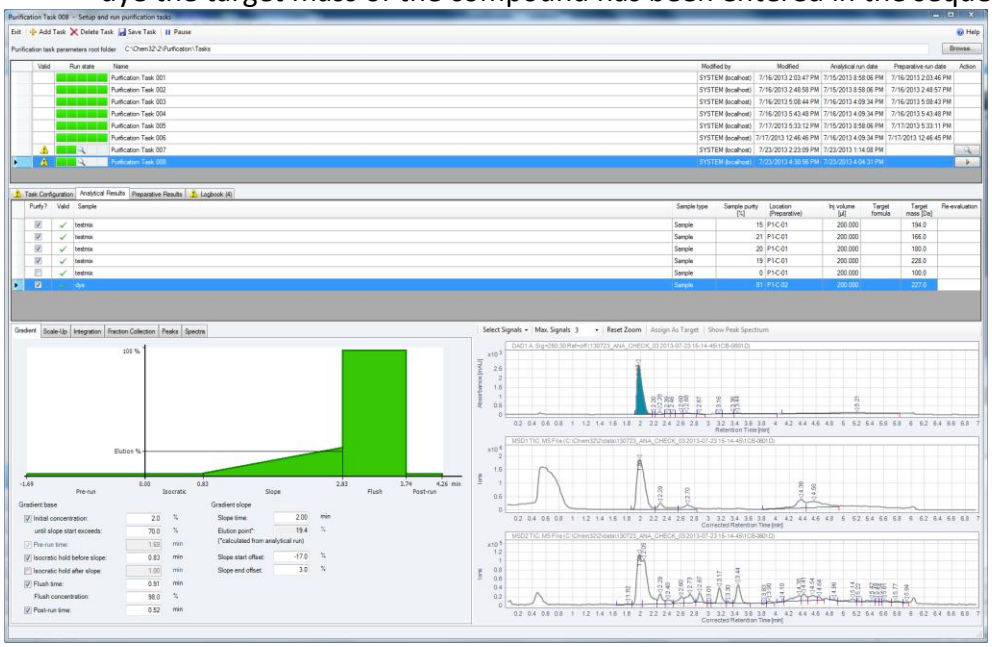
14. Continue in the same way with Ethylparabene (M=166.1), Propylparaben (M=180), Benzylparabene (M=228)

15. Once all target masses have been found and focused gradients have been calculated, the samples will have been flagged and will be purified in the next step.

16. If the user would like to purify a sample regardless of the mass being found, a purify tick-mark will need to be checked manually. The sample will be purified by using a generic gradient and by UV based collection.

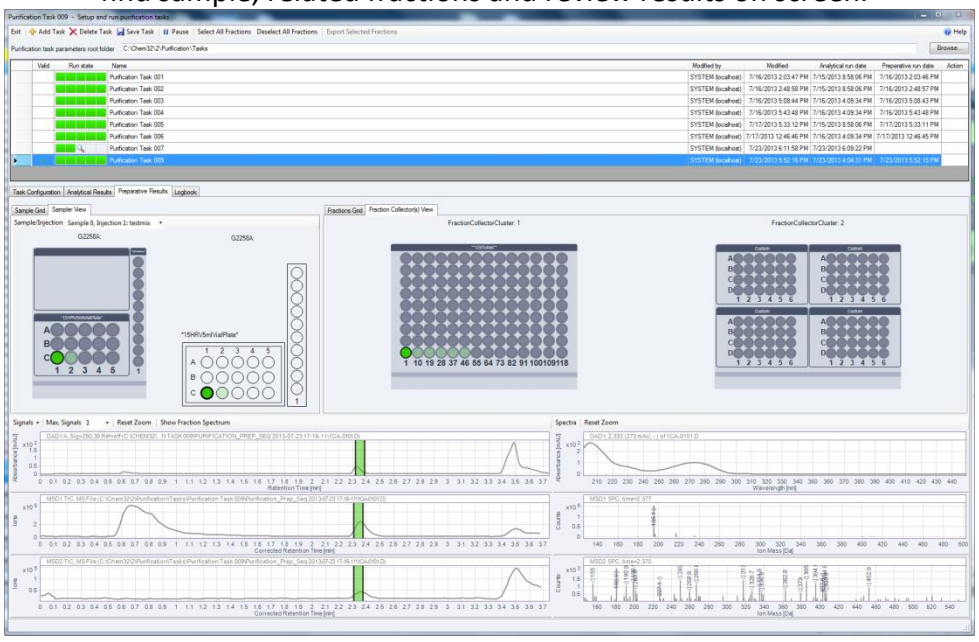


17. To demonstrate automated scale up and fraction collect by using the blue dye the target mass of the compound has been entered in the sequence table.



18. Starting a preparative sequence. Ensure that the system is in operating mode. After pressing the run button again, the system will now start to purify all flagged samples.

19. Check the purification results by navigating to the “Preparative Results” tab, find sample, related fractions and review results on screen.



Note: Target No 1 (Caffeine) has been collected.

20. Continue in the same way with Methylparabene, Ethylparabene, Propylparaben and Benzylparabene.

21. Observe the purification of the blue dye mix (sample 6) and ensure that the blue dye has been collected correctly with a loss of less than 10% into the waste line.
    This will indicate if the right delay volumes and the right threshold and slope parameters have been set.