Application Note

automation for every laboratory

(w) www.eprep-anlytical .com
(e) info@eprep.com.au

ePrep | Application 2022

Automatic Preparation of Samples for Volatile Analysis by P&T GC-MS and for Semi-Volatile Analysis by Direct Injection GC-MS

Pub No. 98-35029 Rev 01

SUMMARY

An automatic ePrep workflow has been developed for the sample preparation of instrument-ready solutions for volatile and semi-volatile analysis. For the volatile samples, a soil extract is aliquoted into a purge and trap (P&T) vial previously aliquoted with a defined volume of water. For the semi-volatile samples, the soil extract is filtered and then prepared in 2mL autosampler vials ready for direct injection by GC-MS.

ePrep Sample Preparation Workstation

INTRODUCTION

The preparation of final solutions ready for US EPA volatile and semi-volatile methods is usually a tedious and manual process. The preparation involves aliquoting extracts from container to container, addition of surrogates and internal standards and final sample preparation ready for the chromatograph whether this be in a purge and trap vial or a 2mL autosampler vial for semi-volatile analysis.

The ePrep is a liquid handling robot which is able to automate manual workflows. Through task-based software, a workflow can be created which will allow minimal training for a laboratory technician to set up the various containers and solutions on the deck and initiate the automated procedure. The workflow will then execute unattended. The ePrep is a syringe-based system which provides features such as the use of sealed containers and the use of organic solvents, without dripping. Volumes from microlitres to millilitres can be aliquoted using patented syringe-change technology. The ePrep is an off-instrument preparation unit thereby allowing final preparation of the chromatography-ready solution in an autosampler rack of the customer's choice.

An automated sample preparation method has been developed and for the analysis of volatile organic compounds contained for example, in the US EPA 8260 method and the same workflow is used to aliquot another extract, through a filtering step, into a 2mL autosampler vial ready for semi-volatile analysis. Before the ePrep automation workflow, soil samples are extracted with 30mL of methanol. The ePrep then enables the removal of human input by automatically aliquoting 100µL of the supernatant into a container which has been filled using the ePrep, with 5mL of deionized water. The whole workflow is carried out using septum-sealed containers.

Each step in the automated workflow will be fully discussed with parameters such as aspiration and dispense rates, pause times of the plunger, priming and washing steps. This will give insight to the technical requirements of the workflow thereby allowing modification to suit a laboratory's specific requirements.

Figure 3. ePrep with filter rack. Luer Syringe with connected Filter

SUMMARY OF VOLATILES AND SEMI-VOLATILES WORKFLOW

Note: soil samples were previously extracted in methanol

Aliquot 5mL of water to P&T vial

↓

Aliquot 100µL supernatant extract to P&T vial

↓

Prepared sample ready for volatiles analysis

Aliquot 2mL of supernatant extract and filter (0.22µm) to an intermediate 2mL autosampler vial

↓

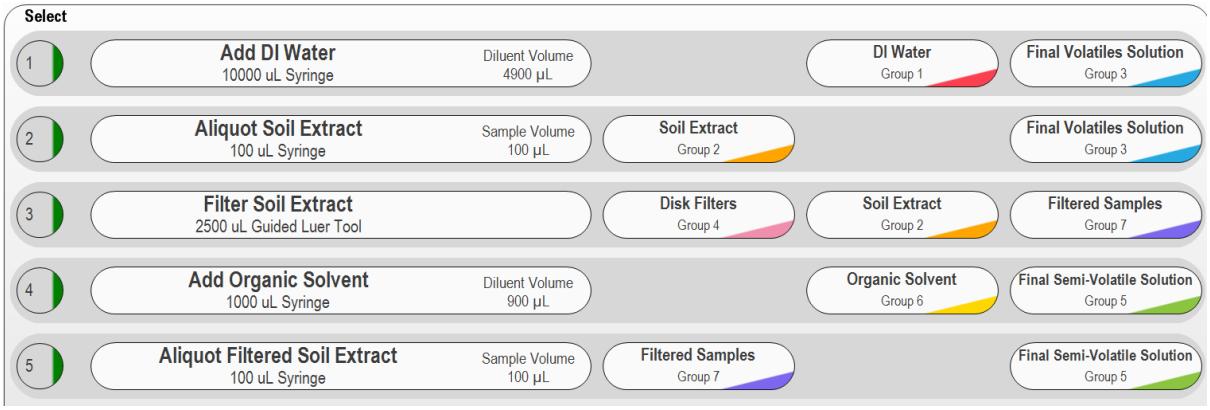
Aliquot 900µL of organic solvent to each of the final autosampler vials

↓

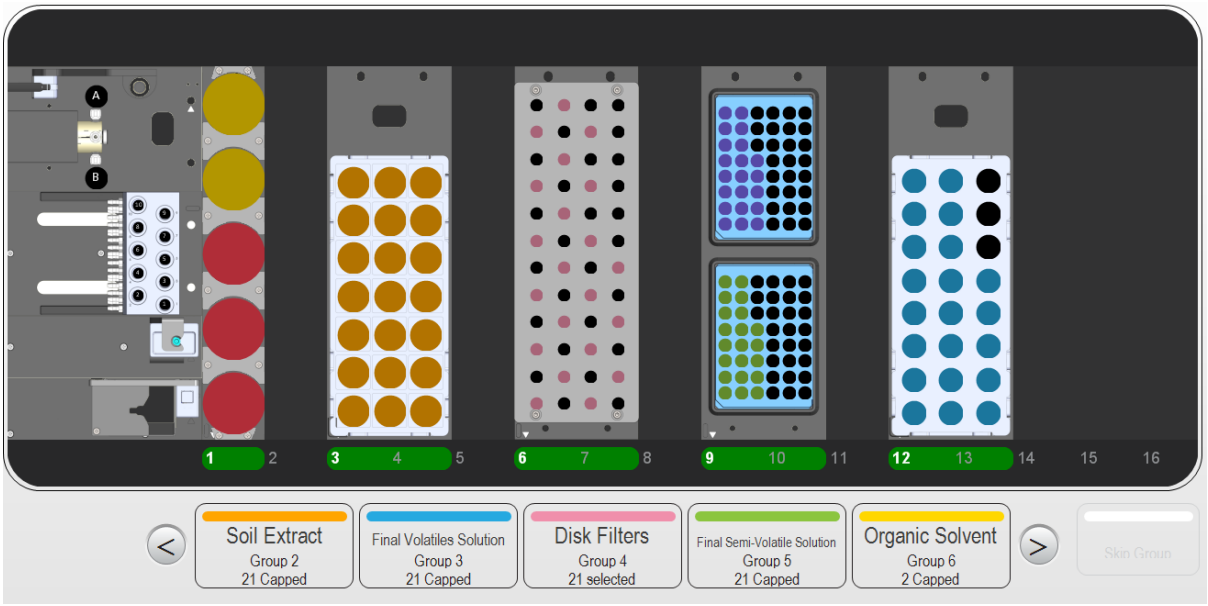
Add 100µL of soil extract filtrate to the final autosampler vial

Prepared sample ready for analysis

Screen capture of the ePrep workflow



Screen capture of the ePrep deck for 21 soil samples (20 samples + 1 blank)



DISCUSSION OF WORKFLOW

This workflow shows the flexibility of the ePrep. Using a soil sample which has been previously extracted off-line using a tumbling technique, the container containing the soil and solvent is transferred to a Belarc rack ready for the ePrep workflow. The first part of the workflow involves preparing the soil extract for volatiles analysis, for example, this could be the US EPA 8260 method. Into a 20mL P&T (purge and trap) vial is aliquoted 5mL of water followed by 100µL of the supernatant of the soil extract. The syringe change between the 10mL syringe used for the water transfer and the 100µL syringe used for the extract transfer occurs seamlessly. It would be easy to include a further addition of an internal standard task if required.

There are many parameters which can be adjusted for syringe priming, washing and aliquoting but in each case, there are default parameters which will be suitable most of the time. For example, for the syringe washing, the wash mode is set to Auto by default which for the addition of the deionized water will result in washing before and after all the water additions have been completed to the 21 vials, but there is no need to wash the syringe between aliquots. Also, by default, the 10mL syringe will aspirate almost the full volume of 10mL syringe (9.8mL) and to save time, will dispense 4.9mL into two vials sequentially.

For the syringe washing of the 100µL syringe used in the second task, because the syringe is aliquoting different soil extract samples, the syringe will be washed between samples with the set Auto mode. The software has in-built smart functionality to realise the syringe will be aspirating from sample to sample. These smart settings and default values associated with the off-the-shelf tasks are a great feature of the ePrep. They allow rapid creation and execution of a workflow without overburdening the lab analyst with decision making along the way. One parameter though that will require changing for this workflow is the default Auto Low setting for the depth of the needle position (located under Advanced Settings) when the extract is aspirated. This is because the soil is still present in the container and if the needle aspirates from the lowest position, there is a very good change the needle would become blocked.

The final volatiles sample now contains 4.9mL of DI water with 100µL of soil extract ready for P&T instrument usually coupled to a GC-MS.

The flexibility of the ePrep, through interchangeable racks on the deck, allow a different sample preparation within the same workflow. We use the same soil extract for the preparation of the chromatograph-ready solution for semi-volatiles analysis, such as the USEPA 8270 method. The ePrep will first filter the extract directly into an intermediate 2mL autosampler vial (step 3 of the workflow). Various filters can be used but, in our case, we used a 0.22µm cellulose acetate filter. The filtering capability of the ePrep is one of the features which set it apart from autopipette systems. The filtering stage involves aspirating the extract solution with a removable needle before the needle is automatically parked and the syringe now containing the extract, picks up the filter.

The filter initially of course will be dry so in the filter settings, is a holdup volume which can be set, and this volume will fill the filter. It was observed the filters can have different hold up volumes so when choosing this parameter, it is best to set a higher volume to ensure the filter has been completely saturated. It also best practice to assume the filtered volume to a tube is not quantitative. So, for this reason rather than filter a defined volume into the final 2mL vial, the supernatant was first filtered into an intermediate vial and then a defined volume was aliquoted from the intermediate vial into the final vial.

The excess volume from this hold up volume step is set to be disposed to the wash station by default but this could be easily changed to dispose this back to the same container or even a different container if desired. A volume of 1mL is then transferred to an intermediate vial.

The needle depth of the aspiration needle of the filter syringe into the supernatant and the aspiration flow rate are important. If the needle is too close to the top of the soil sediment or the aspiration rate is too high, then the soil bed could be disturbed causing the sediment to also be aspirated. Depending on the severity of this, this could then cause blockage of the aspiration needle. The membrane used in the filter was 0.22µm. To ensure no cross-contamination between filtered samples, the filter syringe coupled to the filter syringe aspiration needle was always washed in the wash station after a filtered sample. The wash station will both wash the interior of the syringe and needle and the exterior of the needle.

The make-up volume of 900µL of the organic diluent was first aliquoted to each of the final vials before transfer of the filtrate. As with the aliquoting of the water for volatiles sample preparation, there was no

need to wash the syringe between vials. For our workflow, we used a 1mL syringe for this task but if the time for the workflow needs to be reduced, a 10mL syringe could be used for this task and then the 10mL syringe would be filled for multi-dispense of 900µL volumes. Even though the 900µL is slightly lower than the recommended minimum volume of 10%, there would be very little loss of accuracy and precision if this was done.

It is good practice when aliquoting a sample, to dip the needle into the diluent on dispense to ensure complete transfer of the sample. For this reason, the organic solvent is first dispensed to the 2mL final vials followed by aliquoting the soil filtrate. If this is not done or the needle is above the surface of the liquid, there is a chance that a drop of the filtrate will be left hanging on the needle which will adversely affect quantitative transfer. The lower the dispense volume, the higher the error. The default setting for this parameter is 'Auto Low' for needle depth, for this reason.

The aspirate flow rate for addition of 100µL filtrate to the final vial was set low at 10µL/sec with an aspirate pause time of 4sec. These are critical settings and will dependent on the organic solvent which will be used. If the aspirate flow rate is too high and / or the pause time is too short, cavitation will be seen in the syringe. This occurs because the plunger aspirate speed is too high for the viscosity of the liquid and the liquid will 'lag' behind the plunger tip. The pause time allows time for the liquid to 'catch up' to the plunger. By having a low aspiration rate and high pause time, there will be an extra degree of robustness in the aliquoting of the filtrate. These two settings are not as critical for priming and washing the syringe but especially important when quantitative transfer is a must.

The final semi-volatiles solution is prepared in a 2mL autosampler vial in a rack of your choosing. In this workflow, the filtered sample is diluted 1:10 i.e. 900µL of solvent followed by 100µL of filtered soil extract. This ratio can of course be altered by varying the volumes.

This semi-volatiles part of the workflow is very adaptable and could include the addition of an internal standard step if required. An internal standard task could be added immediately after the 'add 900µL of diluent' step. If 100µL of internal standard solution was added, the diluent volume would need to be reduced to 800µL if the dilution factor was to remain unchanged. Adding the internal standard after the addition of the diluent would ensure that the needle will be dipped into the diluent. Also, the inclusion of the internal standard at this stage of the workflow would save time as the syringe would only need to be washed and primed before the first addition. It would also make sense to dispense 100µL from a 1,000µL syringe so one aspirate would result in 10 dispenses and save workflow time.

RESULTS AND DISCUSSION

A preliminary test of the volatiles sample preparation was carried out. A Laboratory Control Sample (LCS) was prepared by spiking deionized water with a stock standard using the ePrep. Two samples were analysed on two different models of Tekmar P&T trap instruments. The recoveries of all target compounds were calculated by a comparison to a manually prepared LCS and analysed in the batch by P&T GC-MS.

The results are shown in the table below. As can be seen, recoveries are excellent across the entire range of volatile organic compounds.

Table showing recovery of VOCs prepared by the ePrep and compared with a manual preparation technique

Analyte	ATOMX-XYZ		ATOMX	
	Conc.	Recovery	Conc.	Recovery
	µg/L	%	µg/L	%

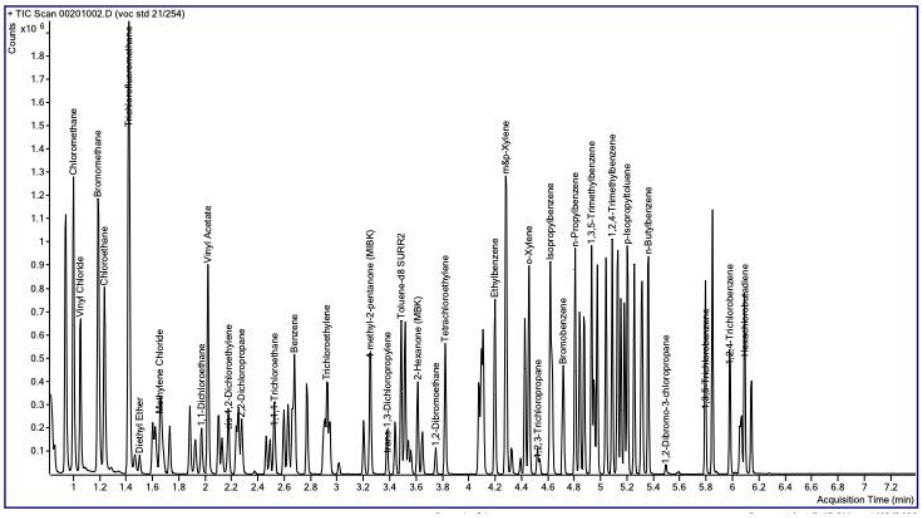
1, 1-Dichloroethylene	47.85	96	48.01	96
1,1,1,2-Tetrachloroethane	48.26	97	45.00	90
1,1,1-Trichloroethane	47.45	95	39.27	79
1,1,2,2-Tetrachloroethane	53.14	106	49.07	98
1,1,2-Trichloroethane	51.61	103	48.90	98
1,1-Dichloroethane	48.15	96	46.60	93
1,1-Dichloropropylene	47.83	96	44.21	88
1,2,3 TrimethylBenzene	50.53	101	53.42	107
1,2,3-Trichlorobenzene	43.86	88	59.50	119
1,2,3-Trichloropropane	53.32	107	48.78	98
1,2,4-Trichlorobenzene	46.82	94	61.64	123
1,2,4-Trimethylbenzene	46.85	94	48.90	98
1,2-Dibromo-3-chloropropane	55.24	110	42.15	84
1,2-Dibromoethane	51.54	103	49.59	99
1,2-Dichlorobenzene	48.43	97	51.96	104
1,2-Dichloroethane	50.25	101	39.02	78
1,2-Dichloroethane-d4 Surr1	53.12	106	51.54	103
1,2-Dichloropropane	50.24	100	49.27	99
1,3,5-Trichlorobenzene	47.14	94	63.31	127
1,3,5-Trimethylbenzene	46.94	94	48.13	96
1,3-Dichlorobenzene	47.80	96	53.04	106
1,3-Dichloropropane	51.12	102	48.69	97
1,4-Dichlorobenzene	48.31	97	51.94	104
2,2-Dichloropropane	48.58	97	41.20	82
2-Butanone (MEK)	553.53	111	518.96	104
2-Chlorotoluene	51.65	103	49.33	99
2-Hexanone (MBK)	568.23	114	461.31	92
4-BFB Surr3	51.00	102	50.21	100
4-Chlorotoluene	48.60	97	50.74	101
4-methyl-2-pentanone (MIBK)	560.65	112	499.26	100
Acetone	560.72	112	555.61	111
Benzene	48.25	97	49.75	100

Bromobenzene	49.38	99	49.16	98
Bromochloromethane	50.35	101	49.83	100
Bromodichloromethane	48.77	98	41.88	84
Bromoform	50.96	102	43.25	87
Bromomethane	466.54	93	482.81	97
Carbon Disulfide	47.15	94	47.12	94
Carbon Tetrachloride	47.57	95	37.35	75
Chlorobenzene	47.22	94	50.72	101
Chloroethane	485.58	97	488.90	98
Chloroform	48.18	96	42.49	85
Chloromethane	483.04	97	505.25	101
cis-1,2-Dichloroethylene	48.11	96	47.29	95
cis-1,3-Dichloropropylene	49.36	99	47.25	95
cis-1,4-Dichloro-2-butene	51.90	104	54.94	110
Dibromochloromethane	50.04	100	42.95	86
Dibromomethane	51.19	102	46.04	92
Dichlorodifluormethane	477.53	96	452.20	90
Diethyl Ether	51.91	104	50.37	101
Ethylbenzene	46.82	94	48.93	98
Hexachlorobutadiene	41.72	83	51.67	103
Iodomethane	44.74	89	53.53	107
Isopropylbenzene	46.13	92	47.68	95
m&p-Xylene	92.17	92	99.70	100
Methyl tert-Butyl Ether (MTBE)	51.85	104	46.40	93
Methylene Chloride	49.29	99	55.57	111
Naphthalene	53.21	106	54.27	109
n-Butylbenzene	45.12	90	51.65	103
n-Propylbenzene	46.87	94	49.94	100
o-Xylene	46.97	94	47.59	95
Pentachloroethane	48.74	97	44.78	90
p-Isopropyltoluene	44.95	90	49.41	99
sec-Butylbenzene	45.47	91	48.64	97

Styrene	47.49	95	51.73	103
tert-Butylbenzene	47.35	95	46.76	94
Tetrachloroethylene	48.03	96	48.82	98
Toluene	47.35	95	48.96	98
Toluene-d8 Surr2	50.94	102	48.47	97
trans-1,2-Dichloroethylene	47.68	95	47.92	96
trans-1,3-Dichloropropylene	50.52	101	46.08	92
trans-1,4-Dichloro-2-butene	53.49	107	47.77	96
Trichloroethylene	47.79	96	44.61	89
Trichlorofluoromethane	482.41	96	412.31	82
Vinyl Acetate	533.18	107	442.62	89
Vinyl Chloride	479.87	96	423.60	85

It should be noted that the purge and trap vials were septum-sealed during the workflow and therefore pierced through the syringe operation. To avoid any losses of volatiles after the ePrep sample preparation, it is highly recommended samples either be analysed immediately or stored under refrigeration before purge and trap GC-MS analysis.

Chromatogram below showing GC-MS analysis after P&T of compounds contained in the US EPA 8260 method



CONCLUSION

The ePrep is able to automate the preparation of samples for volatiles and semi-volatiles analysis from soil extracts. Using the one workflow, samples were prepared ready for volatile analysis by purge and trap GC-

MS. Also, soil extracts were prepared by the ePrep through filtering and then final make up to volume in a 2mL autosampler ready for semi-volatile analysis by GC-MS.

ACKNOWLEDGEMENTS

Australian Laboratory Services (ALS) Melbourne for technical advice and providing the data used within this report.