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Automated Sample Preparation for the Analysis of Estrone by GC Triple Quadrupole Mass Spectrometry

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Introduction

Analysis of endocrine disruptors is increasingly becoming a high volume analysis in many labs and crossing disciplines such as clinical chemistry, industrial exposure, drug discovery and development and environmental analyses including emerging contaminants and persistent organic pollutants. The demand placed on laboratories for these high volume tests places a burden on not only the analytical measurement tools but most importantly accurate and reproducible sample preparation. This poster briefly outlines how the Agilent 7696A Sample Prep WorkBench can be used to prepare samples for analysis through GC/MS/MS using an automated workflow.

The need for accurate analysis of endocrine disruptors (EDCs) is growing in demand. The excretions of the non-metabolized parent drug and its metabolites are often not fully degraded through conventional wastewater treatment processes. Thus, these compounds are found in freshwater bodies such as rivers and transported to aquifers. Due to decades of extensive use, these compounds have become ubiquitous, persistent organic pollutants, and could pose a risk to human health. The need to study their transport and fate in the environment is of paramount importance. This poster illustrates automated sample preparation including preparation of calibrators and derivatization protocol using the 7696A Sample Prep WorkBench for the analysis of a group of known endocrine disruptors by GC-MS/MS.

Figure 1. Agilent 7696A Sample Prep WorkBench.

Experimental

All calibrators and derivatives were prepared on the Agilent 7696A Sample Prep WorkBench and analyzed on the Agilent 7890A/7000B GC/MS. The instrument conditions were determined by Mrozinski, Hernandez-Ruiz and Macherone (2013) and illustrated below in Table 1.

Table 1. GC/MS conditions.

GC Run Conditions	
Analytical columns	Column 1: Agilent HP-5MS UI 15 m x 0.25 mm x 0.25µm (P/N 19091S431UI)
	Column 2: Agilent HP-5MS UI 15 m x 0.25 mm x 0.25µm (P/N 19091S431UI)
Injection volume	2 µL
Injection mode	Cold, split-less using Multi-Mode Inlet (MMI)
Inlet temperature	70 °C for 0.01 min
	450 °C/min to 280 °C for 3 min
Gas saver	On: 20 mL/min after 3 min
Purge flow	30 mL/min at 1.5 min
Cryo	On
Cryo use temperature	72 °C
Fault detection	30 min
Timeout detection	On: 10 min
Oven temperature	120 °C for 0.5 min
	40 °C/min to 240°C, hold for 0 min
	5 °C/min to 280°C, hold for 3.75 min
Carrier gas	Helium in constant flow mode
	Column 1: 0.8 mL/min; Column 2: 1.0 mL/min
Average velocity	23.498 cm/sec
Transfer line temp	280°C
Run Time	15.25 min
MS conditions	
Tune	atunes.eiex.tune.xml
Gain Factor	50
Acquisition parameters	Multiple reaction monitoring (MRM)
Collision gas	1.5 mL/min nitrogen
Quench gas	2.25 mL/min helium
Solvent delay	6.0 min
MS temperatures	Source 300°C; Quadrupoles 150°C

Time Segment	Start Time	Name	Precursor Ion (m/z)	Product Ion (m/z)	Dwell (ms)	Collision Energy (V)
1	10.5	E1	342.0	257.0	150	15
1	10.5	E1	342.0	244.0	150	15

Sample Preparation

Estrone (E1), BSFTA / TCMS (99% / 1%), anhydrous acetonitrile and anhydrous pyridine were purchased from Sigma-Aldrich (USA). Stock solutions of E1, E2, and EE2 were prepared in anhydrous acetonitrile and used to create a working mixture required for calibrator preparation.

Trinh et al. (2011) have demonstrated an E1 MDL near 1.0 ng L^{-1} taking into consideration a 1000-fold concentration when samples are prepared (1.0 L sample volume concentrated to 1.0 mL). A stock solution of E1 was prepared in anhydrous acetonitrile and used to create a working mixture required for calibrator preparation. For this evaluation calibrators were prepared at 1.0, 2.5, 5.0, 10.0, and 50.0 ng/mL using the 7696A Sample Prep WorkBench.

For the derivatization, a stock reagent of 10/10/80% (v/v) BSTFA+TCMS/anhydrous pyridine/anhydrous acetonitrile was prepared and added to the dried calibrators and heated to 60°C for 30 minutes by the 7696A Sample Prep WorkBench.

Results and Discussion

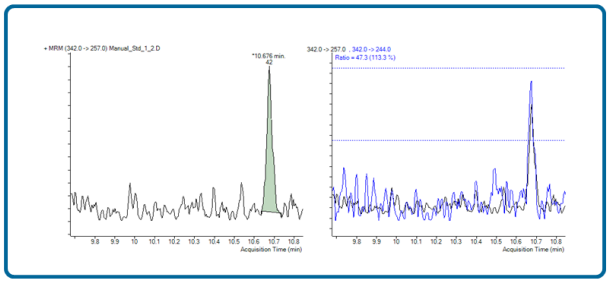
Workbench sample preparation

Automation using the workbench significantly reduces analyst time spent on sample preparation, removes the potential for sampling errors while maintaining the recovery and precision achieved through manual work up. In this study, a recovery of 113.37% was determined at the 1.0 ng/mL (1 pg on column) level with three replicate injections and an average precision of 5.162% RSD (%RSD range 3.32 – 6.89) over the five levels. Table 3 illustrates these results. Figure 2 illustrates the quantitative and qualitative SRMs for E1 at 1.0 ng/mL or 1 pg mass on column.

In Figure 2 above, Panel A shows the quantitative MRM 342->257. Panel B shows the qualitative transition 342->244. The dotted lines in B represent the allowable uncertainty for qualifier ratio. Noise region for S/N calculation is 10.4 to 10.6 minutes.

Results and Discussion

Figure 2. Quantifier (A) and qualifier (B) MRMs for estrone at 1.0 pg on column.



GC-MS/MS analysis

For the study defined herein, three replicate injections were made at 5 concentration levels ranging from 1.0 ng/mL to 50.0 ng/mL. Figure 3 illustrates the resulting calibration curve with a correlation coefficient of R^2 = 0.996 for the fifteen total injections.

Figure 3. Calibration curve.

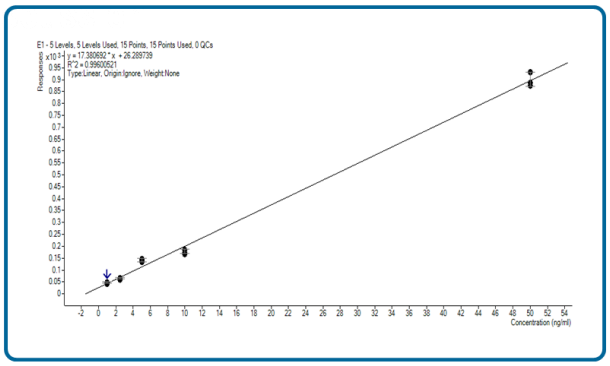


Table 2 shows signal-to-noise (S/N) and percent recovery at 1.0 ng/mL (1 pg on column) and Table 3 shows percent RSD for three replicate injections at five calibrator levels.

Table 2. S/N and % recovery at 1.0 pg on column

	Sample		E1 Method		E1	
Name	Type	Level	Exp. Conc.	Area	Final Conc.	S/N
Std_1_1	Cal	1	1.0 ng/mL	48.18	1.29	11.20
Std_1_2	Cal	1	1.0 ng/mL	42.01	0.94	9.00
Std_1_3	Cal	1	1.0 ng/mL	45.97	1.17	12.40
		% Recovery			113.37	

Results and Discussion

Table 3. Area %RSD for 3 replicate injection over at levels.

Name	Sample Type	Level	Exp Conc.	E1 Area
Std_1_1	Cal	1	1	48.18
Std_1_2	Cal	1	1	42.01
Std_1_3	Cal	1	1	45.97
		% RSD		6.89
Std_2_1	Cal	2	2.5	65.86
Std_2_2	Cal	2	2.5	65.75
Std_2_3	Cal	2	2.5	59.74
		% RSD		5.49
Std_3_1	Cal	3	5	134.20
Std_3_2	Cal	3	5	147.65
Std_3_3	Cal	3	5	137.09
		% RSD		5.07
Std_4_1	Cal	4	10	184.80
Std_4_2	Cal	4	10	167.32
Std_4_3	Cal	4	10	173.81
		% RSD		5.04
Std_6_1	Cal	5	50	931.48
Std_6_2	Cal	5	50	874.49
Std_6_3	Cal	5	50	887.74
		% RSD		3.32

Instrument Detection Limit

Wells et al (2011) state that when the sample set is less than thirty, the one-tail Students-t distribution can be used to estimate the instrument detection limit (IDL). For 99% confidence and n-1 degrees of freedom, the Students-t Table value for this study is 6.965. Substitution of 6.965 and 6.89 %RSD for the low calibrator into the IDL equation (Equation 1) results in an estimated instrument detection limit of 0.48 pg E1 on column. This value is in fair agreement with Trinh et al (2011) who determined MDLs of 0.7 ng L^{-1} with 99% confidence and n=7 replicates.

Equation 1. Estimated instrument detection limit (IDL) based on area % RSD for 1.0 ng/mL calibrators (n=3)

$$IDL_{\%RSD} = \frac{(6.965 * 6.89\% * 1.0pg)}{100} = 0.48pg$$

Conclusions

The Agilent 7696A Sample Prep Workbench can be used to accurately prepare samples, calibrators and QC's for the analysis of estrogenic and other endocrine disruptors in an automated workflow that includes on board derivatization. This poster illustrates the effectiveness of automating sample derivatization followed by analysis via GC triple quadrupole mass spectrometry. Excellent recoveries and precision were obtained over the calibration range and an instrument detection limit was determined in good agreement with MDLs reported in the literature.

References

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