Using the Agilent 7696A Sample Prep WorkBench for the analysis of estrone by GC Triple Quadrupole Mass Spectrometry

Application Note

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Abstract

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 contaminate 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 application note 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.

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Introduction

The need for accurate analysis of endocrine disruptors (EDCs) such as estrogens, androgens, progestins, corticosteroids, and glucocorticoids in ground, surface, and potable water sources is growing in demand. The major source of these compounds in the environment is an iatrogenic artifact of hormonal therapies for agricultural livestock and humans. The excretions of the nonmetabolized 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. Adverse effects even at ppt levels include, but are not limited to, abnormal population ratios of male to female in fish and amphibian communities, reversible feminization of fish species, inhibition of reproduction pathways, morphological changes such as an increased occurrence of hermaphroditism, and disruption of normal pheromone responses. 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 application note 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.

Experimental

Standards and Reagents

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

Instruments

The Agilent 7696A Sample Prep WorkBench was used to prepare calibration standards and perform automated derivatization of the analytes. The measurement experiments were performed on an Agilent 7890A Series GC equipped with a multimode inlet (MMI) in cold split-less injection mode and an Agilent 7693A 150 position auto-sampler coupled to an Agilent 7000B Triple Quadrupole GC/MS in EI mode. The instrument conditions are listed in Tables 1 and 2.

Table 1. GC/MS Conditions

GC run conditions	
Analytical columns	Two 15 m HP-5MS UI, (p/n 19091S431UI) connected sequentially using the Agilent Purged Ultimate Union (p/n G1472A)
Injection volume	2 µL
Injection mode	Cold, split-less using Multi-Mode Inlet (MMI)
Inlet temperature	70 °C for 0.01 minutes 450 °C/min to 280 °C for 3 minutes
Gas saver	On 20 mL/min after 3 minutes
Purge flow	30 mL/min at 1.5 minutes
Cryo	On
Cryo use temperature	72 °C
Fault detection	30 minutes
Timeout detection	On 10 minutes
Oven temperature	120 °C for 0.5 minutes 40 °C/min to 240°C, hold for 0 minutes 5 °C/min to 280°C, hold for 3.75 minutes
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 temperature	280 °C
Run time	15.25 minutes
MS conditions	
Tune	atunes.eiex.tune.xml
Gain factor	50
Acquisition parameters	Multiple reaction monitoring (MRM)
Collision gas	1.5 mL/min
Quench gas	2.25 mL/min
Solvent delay	6.0 minutes
MS temperatures	Source 300 °C Quadrupoles 150 °C

Table 2. MRM Parameters

Time segment	Start time	Compound 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 using the Agilent 7696A Sample Prep WorkBench

Trinh *et al* (2011) have demonstrated an E1 MDL near 1.0 ng L-1, taking into consideration a 1,000-fold concentration (1.0 L sample volume concentrated to 1.0 mL). For this evaluation, five 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 also by the 7696A Sample Prep WorkBench.

Results and Discussion

7696A Sample Prep WorkBench sample preparation

Automation using the 7696A Sample Prep 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 application note, a recovery of 133.37% 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 (range 3.32–6.89) over the five levels. Tables 3 and 4 illustrate these results. Figure 1 illustrates the quantitative and qualitative SRMs for E1 at 1.0 ng/mL or 1 pg mass on column.

Table 3. Low Calibrator S/N and % Recovery at 1.0 ng/mL (1 pg on Column)

Name	Sample type	Level	E1 method Exp. conc.	Area	E1 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	

Table 4. Calibrator %RSD (5 Levels, n = 3 Replicates)

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

GC/MS/MS analysis

For this study, three replicate injections were made at five concentration levels ranging from 1.0 ng/mL to 50.0 ng/mL. Figure 2 illustrates the resulting calibration curve with a correlation coefficient of $R^2 = 0.996$ for the 15 total injections.

Instrument Detection Limit

Wells *et al* (2011) state that, when the sample set is less than 30, 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 IDL 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.

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

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

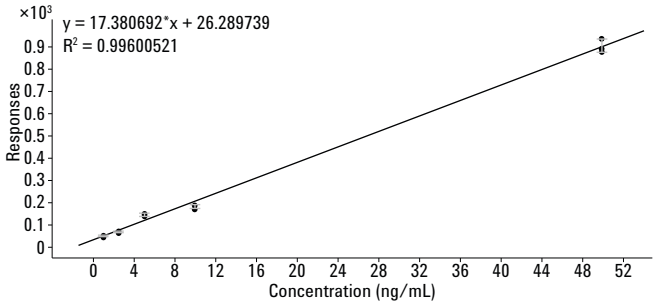
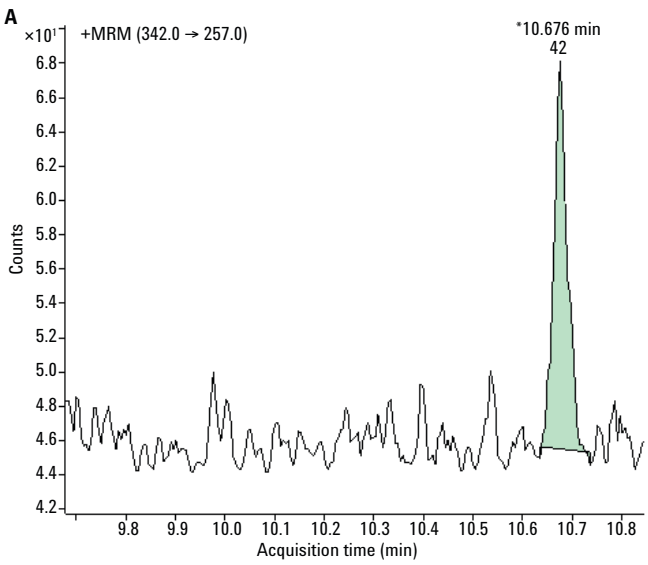


Figure 2. E1 Calibration curve: three replicate injections at five levels.



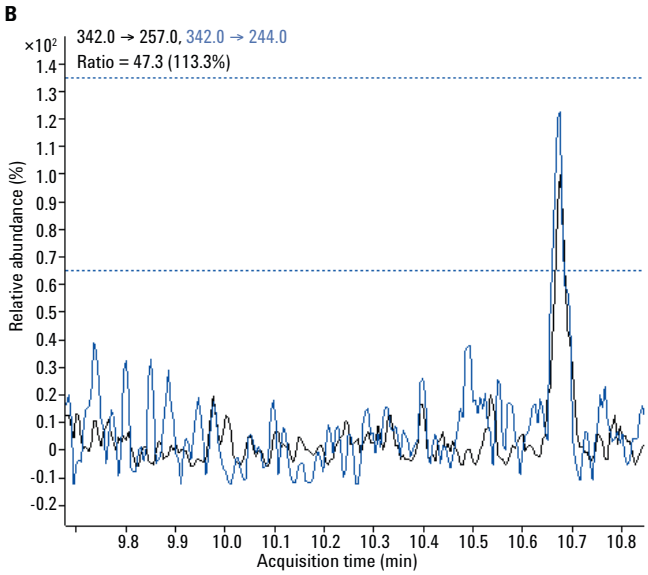


*Figure 1. 1.0 ng/mL EI. **A** shows the quantitative SRM 342 → 257. **B** shows the qualitative transition 342 → 244. The dotted lines in **B** represent the allowable uncertainty for qualifier ratio. Noise region for S/N calculation is 10.4 to 10.6 minutes.*

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 application note illustrates the effectiveness of automating sample derivatization followed by analysis using GC triple quadrupole mass spectrometry. Excellent recoveries and precision were obtained over the calibration levels and an IDL was determined in good agreement with MDLs reported in the literature.

References

1. Trinh T., *et al.* "Simultaneous determination of estrogenic and androgenic hormones in water by isotope dilution gas chromatography–tandem mass spectrometry". *J. Chrom A*, 1218 (2011) 1668–1676.

2. Wells G, Prest H, Russ CW. "Why use signal-to-noise as a measurement of MS performance when it is often meaningless". Agilent Application Note 5990-8341EN. (2011) Agilent Technologies, Inc.

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