Large-Volume Injection (LVI)-LC/MS/MS for Trace Level Detection of Illicit and Prescribed Drugs in Municipal Wastewaters

Application Note

Environmental

Authors

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Abstract

Commercially available autosampler hardware from Agilent was used to upgrade an Agilent 1100 Series HPLC in order to eliminate the need for offline or online solid phase extraction. Using a 1800 µL large-volume injection (LVI) sample in conjunction with liquid chromatography and tandem mass spectrometry (LC/MS/MS), selective illicit drugs and their metabolites were analyzed in municipal (raw influent) wastewaters. This application note demonstrates good linearity for analytes from 9 pg to 450 ng on Agilent's ZORBAX Eclipse Plus C18 column. High accuracy, recovery, and precision were obtained without compromising sensitivity.

Agilent Technologies

Introduction

Sewage surveillance is gaining attention as a cost-effective tool in supplementing efforts to identify drug abuse patterns in communities [1]. Researchers report quantifiable amounts of illicit and/or prescription drugs in raw influent and treated effluent of wastewater treatment plants (WWTPs), drinking, and recreational waters. Due to low level occurrence of the analytes in these matrices, current analytical protocols include sample pretreatment and concentration steps such as solid phase extraction (SPE). However, the major disadvantages of SPE are that the technique is time-consuming, labor-intensive, and requires large sample volumes ranging from 50-1000 mL. Despite the advantage of having a good sample cleanup and concentration, the SPE methodology is known for low and variable recovery of analytes, lowered enrichment factor due to low volume injection, and contamination of analytes with SPE materials [2].

This application note describes the technique of large-volume injection (LVI) with LC/MS/MS for trace level detection of a suite of analytes ranging from prescription medicines to street drugs, and their metabolites in wastewater influent.

LVI consists of the direct injection of samples with volumes ranging up to 2000 µL or greater, which is large compared to conventional injection volumes of 5-20 µL. LVI works on the principle concentrating analytes onto the head of the analytical column during injection of a low-elutropic strength sample. Salts and other matrix components that do not partition into the stationary phase pass unretained through the column. Following analyte concentration on the analytical column, which is analogous to SPE, an increase in the elutropic strength of the mobile phase promotes elution and separation of the concentrated analytes. The major advantages of LVI include an increase in sensitivity while maintaining accuracy and precision with minimal sample handling and small sample volume (such as 2 mL). Large-volume injection approaches are robust for routine analysis of polar organic contaminants such as fluorochemicals [2, 3], illicit drugs and related substances in municipal wastewater [4].

LVI was optimized and evaluated by examining matrix effects, accuracy, and precision through standard addition experiments. The detection and quantification limits of the instrument and method were then determined using these optimized conditions. In addition, the stability of samples under storage conditions was evaluated. Finally, the analytical method was applied to 24 hr, flow-normalized composite samples of raw influent collected from a single WWTP in order to determine the levels of illicit drugs, and their metabolites, in a selected community on a particular day. The chemical structures of the nine analytes observed in the study sample are presented in Figure 1.

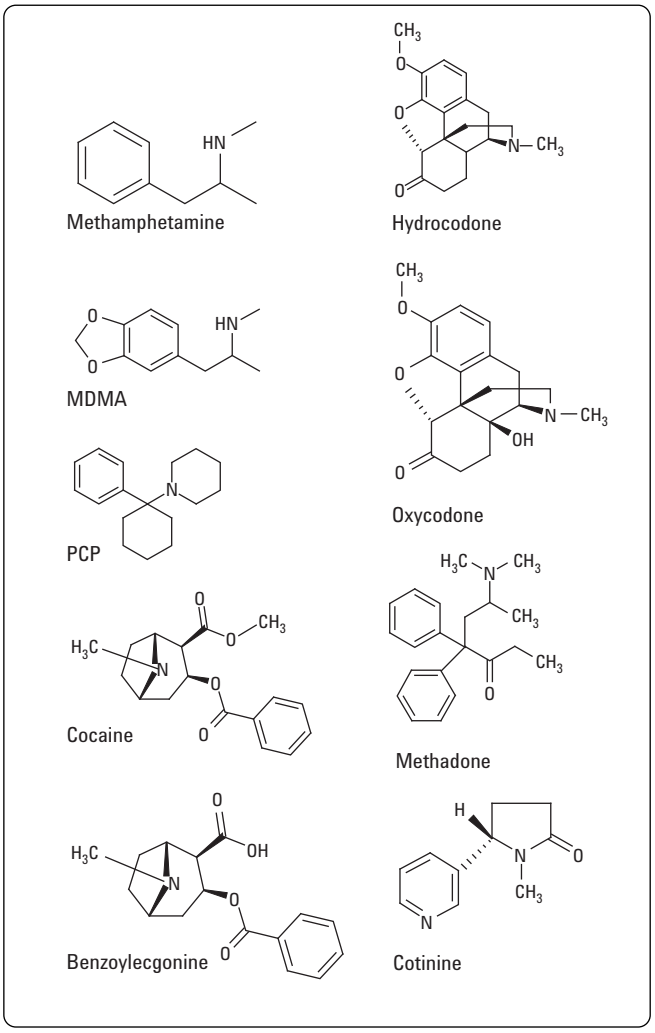

Figure 1. Chemical Structures of the Compounds Under Study

Experimental

Reagents

Reagent-grade glacial acetic acid, EMD Chemicals (Gibbstown, NJ, USA).

HPLC-grade acetonitrile and methanol, Fisher Scientific (Fair Lawn, NJ, USA).

Standards

Drugs of interest (analytical grade >99%, 1 mg/ml in methanol or acetonitrile) purchased from Cerilliant Corporation (Round Rock, TX) include:

1. (±) methamphetamine, (±) 3,4-methylenedioxymethamphetamine (MDMA), phencyclidine
2. cocaine, benzoylecgonine
3. (±)-methadone, hydrocodone, oxycodone,
4. cotinine

Deuterated internal standards (analytical grade >99%, 100 µg/ml in methanol or acetonitrile) were purchased from Cerilliant Corporation (Round Rock, TX, USA):

1. methamphetamine-D_5, (±) 3,4-methylenedioxy-methamphetamine-D_5 ((±)MDMA-D_5), phencyclidine-D_5
2. cocaine-D_3, benzoylecgonine-D_3(±)
3. methadone-D_9, hydrocodone-D_6, oxycodone-D_3
4. cotinine-D_3

Sample Collection and Preparation

1. A 15-mL raw wastewater aliquot of a 24 hr flow-normalized composite was collected in a sterile, polypropylene plastic tube from a local WWTP and stored at -80 °C.
2. Frozen samples were brought to room temperature and a 7-mL aliquot volume was centrifuged in an IEC clinical centrifuge (Thermo IEC, Nutley, NJ) for 30 min at a maximum speed of 7100 rpm (5125 g).
3. After centrifugation, the supernatant was transferred into a 6-mL autosampler glass vial and spiked with deuterated internal standards. All samples were analyzed within 24 hrs of preparation.

LC/MS/MS Instrumentation

Large-volume injections of 1800 µL were made possible by installing the 900-µL injection upgrade kit (Agilent p/n G1363A) that includes a 900-µL analytical head, 900-µL stainless steel loop extension (Agilent p/n G1313-87303), 900-µL needle (Agilent p/n G1313-87202), and 1,400-µL extended seat loop (Agilent p/n G1313-87308) installed between the seat capillary fitting and injection port 5 of the analytical head (Figure 2). Autosampler vials (6 mL) (p/n 9301-1377), caps (p/n 9301-1379), septa (p/n 9301-1378) and the corresponding trays (Agilent p/n G1313-44503) were also installed (Figure 3).

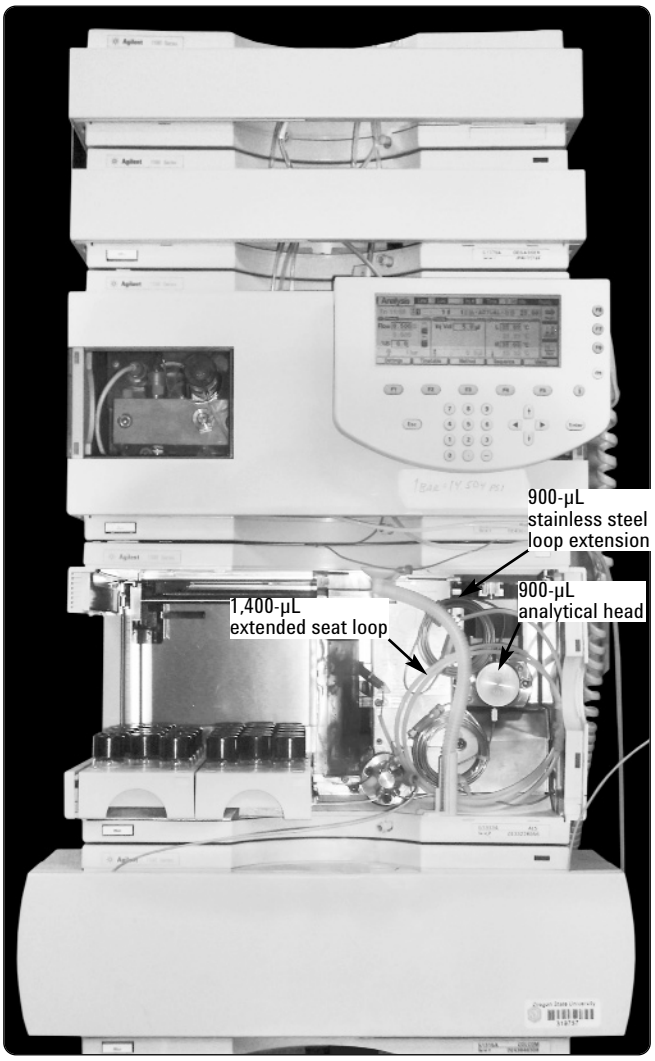

Figure 2. An Agilent 1100 HPLC system with a large-volume injection upgrade kit installed in the G1313A ALS (cover removed).

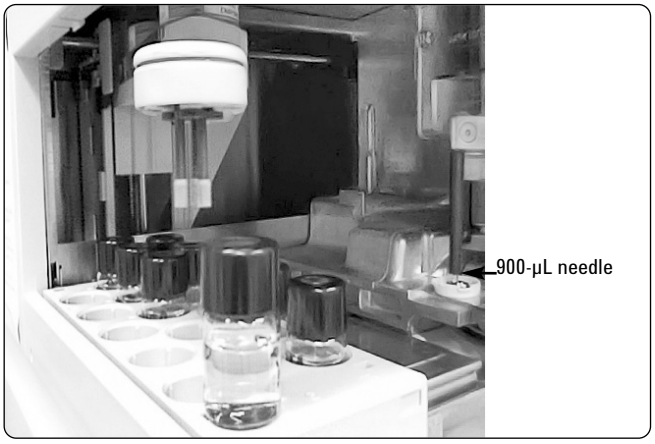

Figure 3. Large-volume autosampler vials (6 mL) with matching tray.

The LC was interfaced to a triple quadrupole mass spectrometer and the multiple reaction monitoring (MRM) parameters are listed in Table 1.

Table 1. MRM Settings for Quantifier Ion of Selected Drugs and Internal Standards. Qualifier Ions and Parameters are Presented in Parentheses.

Analyte	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Cone (V)	CE[a] (E)
1. Methamphetamine	150.0	119.2 (91.1)	20 (25)	10 (15)
2. MDMA	194.1	163.4 (105.1)	25 (30)	10 (25)
3. Phencyclidine	244.2	159.4 (86.2)	20 (20)	10 (10)
4. Cocaine	304.1	182.3 (105.1)	30 (35)	20 (25)
5. Benzoylecgonine	290.2	168.4 (105.1)	20 (30)	20 (20)
6. Hydrocodone	300.2	199.4 (171.3)	35 (40)	30 (40)
7. Methadone	310.3	265.5 (105.1)	25 (25)	20 (25)
8. Oxycodone	316.2	298.5 (241.1)	25 (25)	20 (30)
9. Cotinine	177.1	98.1 (80.0)	30 (30)	20 (20)
Internal Standards				
Methamphetamine-D_5	155.1	91.7	20	20
MDMA-D_5	199.2	165.2	25	10
Phencyclidine-D_5	249.4	164.4	15	15
Cocaine-D_3	307.3	185.5	30	20
Benzoylecgonine-D_3	293.2	171.4	30	20
Hydrocodone-D_6	306.3	202.4	45	30
Methadone-D_9	319.4	268.5	25	20
Oxycodone-D_3	319.3	301.5	25	20
Cotinine-D_3	180.2	80.1	25	25

[a] Collision energy

LC Conditions

Column: Agilent ZORBAX Eclipse Plus C18 Rapid Resolution, 4.6 × 150 mm, 3.5 µm (p/n 959963-902)

Guard column: Agilent ZORBAX Reliance cartridge guard column (12.5 × 4.6 mm i.d.) (p/n 820950-936)

Column temperature: 35 °C

Mobile phase: A=0.1% acetic acid in 5% methanol
B=100% acetonitrile

Column flow rate: 0.5 mL/min

Gradient:

Time (min)	%B
0-9	5
9-13	5 to 20
13-23	20
23-27	20 to 100
27-29	100
29-30	100 to 5
30-35	5

Injection volume: 1,800 µL

Needle wash: 5% formic acid in isopropanol

Injection program

Step	Function	Parameter
1	Wash	Vial location
2	Draw	900 µL sample
3	Eject	900 µL seat
4	Draw	900 µL sample
5	Inject	
6	Wait	9.00 min
7	Value	Bypass

MS Conditions

Mode: Positive ESI

Nebulizer: Nitrogen (350 psi)

Collision gas: Argon

Source temperature: 150 °C

Desolvation gas temperature: 450 °C

Capillary voltage: 3.57 kV

Extractor voltage: 3 V

RF lens voltage: 0.2 V

Cone gas flow: 25 L/h

Desolvation gas flow: 650 L/h

Ion energy 1 and 2: 0.5 V and 2.0 V

Entrance and exit potential: 11 V and 25 V

Results and Discussion

During automated injection programming, the injector is initially set to "main pass" mode so that the sample is transferred to the analytical column (Figure 4). After the sample is loaded onto the analytical column, the system is switched to "bypass" mode so that the mobile phase/gradient bypasses the large volume of the injector sample loop. The time it takes the gradient to get from its formation point to the head of the column is reduced, minimizing chromatographic run times (Figure 4). Chromatographic separation was achieved using the Agilent ZORBAX Eclipse Plus C18 Rapid Resolution column (Figure 5).

Eight-point calibration curves were obtained daily from freshly prepared standards in deionized water. Linear regressions with R^2 values greater than 0.99 were obtained with 1/X-weighting and were not forced through the origin.

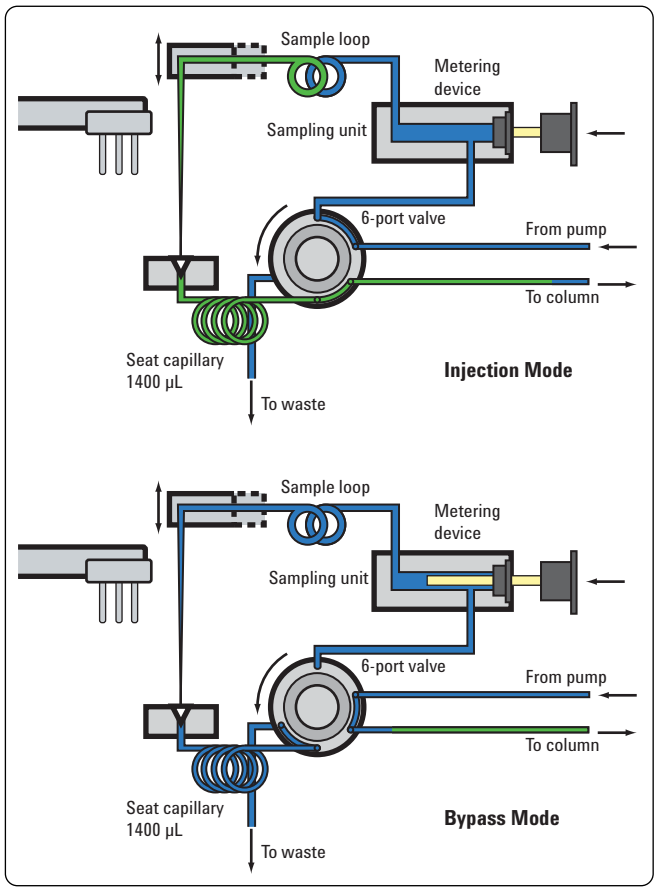

Figure 4. Schematics showing large-volume injection operation.

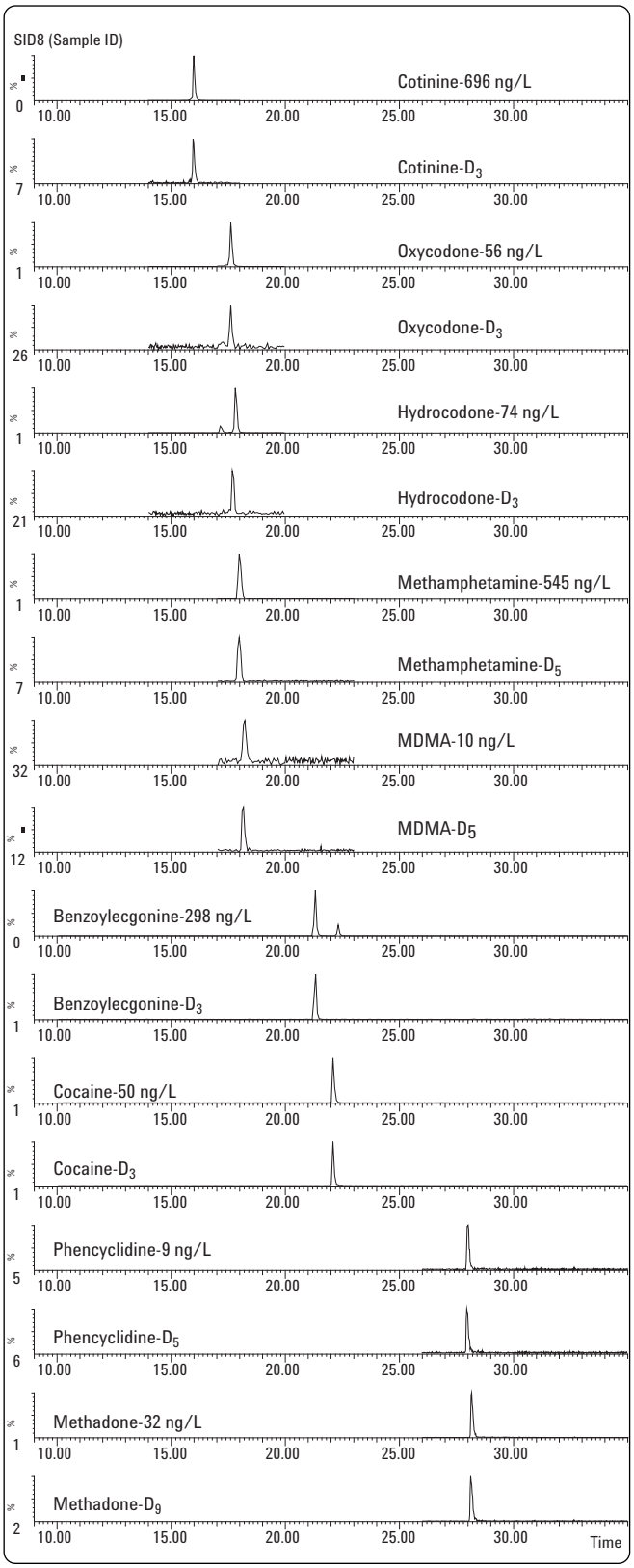

Figure 5. MRM chromatograms and concentrations of the selected drugs present in the study wastewater.

Validation

1. Mean concentrations in unspiked wastewater samples obtained from solvent-based calibration curves were statistically equal at the 95% confidence interval (CI) to those determined by standard additions (Table 2). The stable-isotope internal standards compensated for matrix effects in wastewaters and indicate that the analyte concentrations can be determined directly from calibration curves obtained from standards prepared in deionized water.
2. The instrument detection limits (IDL) ranged from 1.5-5.0 ng/L and the instrumental limits of quantification (LOQ) ranged from 5-250 ng/L (Table 2).
3. Intraday and interday precision, as indicated by % relative standard deviation (RSD), ranged from 2-11% (Table 2).
4. No carryover effects were observed in blanks containing internal standards made in deionized water.
5. Raw area counts of the deuterated internal standards were similar in blanks and wastewater samples, suggesting minimal matrix effects.

Table 2. Instrument Detection Limits (IDL), Limits of Quantification (LOQ), Linearity, Accuracy, Recovery, and Precision for LVI-LC/MS/MS

			Wastewater Concentration (ng/L) Mean ± 95% CI		Precision (%RSD)	
Analyte	**IDL (ng/L)**	**LOQ (ng/L)**	**Solvent Based Calibration**	**Standard Addition**	**Intra-day**	**Inter-day**
Methamphetamine	1.5	10	550 ± 11	549 ± 5	7	5
MDMA	2.5	5	9.5 ± 0.5	9.6 ± 0.2	5	7
PCP	2.5	5	9.7 ± 0.5	9.7 ± 0.1	5	9
Cocaine	2.5	5	48 ± 3	49 ± 1	3	6
Benzoylecgonine	1.5	10	288 ± 9	284 ± 3	2	5
Hydrocodone	2.5	10	72 ± 3	70 ± 1	9	11
Oxycodone	2.5	10	54 ± 3	54 ± 1	6	8
Methadone	2.5	5	32 ± 2	29 ± 1	5	8
Cotinine	5.0	250	698 ± 27	703 ± 7	6	8

Conclusions

This application has shown that large-volume injection is a time-saving technique when combined with LC/MS/MS for the analysis of illict and legal drugs in wastewaters. Despite injecting large volumes of wastewater, fouling of the mass spectrometer was not a problem nor was the column lifetime reduced. Accuracy and reproducibility were acceptable under EPA criteria. Sensitivity and specificity were maintained. Matrix effects were compensated by internal standards.

Acknowledgments

The authors truly appreciate the technical contributions by Lenore Kelly, Dawn Fulton, John Puryear, John W. Henderson, Jr., and Doug Martin of Agilent.

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Printed in the USA
April 10, 2012
5990-4622EN

Agilent Technologies