

# Laser Diode Thermal Desorption/Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry Analysis of Selected Steroid Hormones in Wastewater: Method Optimization and Application

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A rapid and reliable method enabling high-throughput sample analysis for quicker data generation, detection, and monitoring of eight selected steroid hormones in water matrixes was developed and validated. Our approach is based on a novel sample introduction method, the laser diode thermal desorption/atmospheric pressure chemical ionization (LDTD/APCI) coupled to tandem mass spectrometry (MS/MS). The optimization of instrumental parameters and a method application are presented. Our method was successfully applied to spiked effluent wastewater in the low-nanogram per liter concentrations with total analysis time reduced to seconds (15 s) using LDTD/APCI-MS/MS compared to minutes with traditional liquid-chromatography coupled to tandem mass spectrometry (LC-MS/MS) following solid-phase extraction (SPE). The instrumental detection limits for LDTD/APCI-MS/MS ranged from 5 to 24  $\mu\text{g L}^{-1}$  and from 13 to 43  $\text{ng L}^{-1}$  for the method detection limits. Calibration curves in wastewater matrix showed good linearity ( $R^2 > 0.99$ ), and precision (intraday and interday) was below 20%. This work demonstrates that LDTD/APCI-MS/MS could be used for fast and effective quantitative analysis of emerging contaminants in different water matrixes with reduced cost by eliminating the chromatography step used in traditional LC-MS/MS.

Monitoring of emerging contaminants (ECs) in the aquatic environment is progressively becoming a priority for government agencies and regulatory agencies as well as the general public. Among the various compounds considered as ECs, there has been growing concerns toward endocrine-disrupting chemicals (EDCs), such as steroid ovarian hormones, that affect the reproductive physiology of wildlife populations with possible implications in human reproductive health as well.<sup>1</sup> Estrogens and progestogens,

from naturally occurring (e.g., estradiol excreted from mammals urine) and synthetic (oral contraception and livestock-farming) sources, have both been detected in waste and surface water matrixes.<sup>2–5</sup> To date, numerous analytical procedures have been developed to identify and quantitate steroid hormones in water matrixes and often include the use of chromatography (liquid or gas) coupled to tandem mass spectrometry (MS/MS). Preconcentration and purifying processes, such as solid-phase extraction (SPE) or liquid–liquid extraction (LLE), are necessary because of matrix complexity and the low-nanogram per liter levels at which they have been reported in the aquatic environment.<sup>6</sup> As a result, the identification and quantification of steroid hormones can be time-consuming, costly, and often result in slow turnover. Although sample preparation steps are time-consuming for steroid hormone analysis in water matrixes, the liquid chromatographic (LC) step also requires several minutes, increasing the overall analysis time and data generation.

Several alternative techniques have eliminated the use of an LC step prior to detection, reducing analysis time, sample pretreatment, and cost, by eliminating column usage and reducing solvent consumption, while increasing sample throughput, such as direct analysis in real time (DART),<sup>7</sup> desorption electrospray ionization (DESI),<sup>8</sup> and atmospheric pressure matrix-assisted laser desorption/ionization (AP MALDI)<sup>9</sup> all coupled to MS/MS. Tandem mass spectrometry is recognized for its ability to quantitate and confirm the presence of selected target compounds in complex mixtures<sup>10,11</sup> with excellent selectivity and specificity. However, to the best of our knowledge, none of these innovative

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ionization sources coupled to MS/MS have been applied to the quantification of ECs in environmental matrixes. Therefore, the need for an environmental screening and/or monitoring method that is both fast and cost-effective is relevant.

This work presents the use of a sensitive method enabling high-throughput sample analysis of eight selected steroid feminizing hormones (Supporting Information Figure S-1) in effluent wastewater using a novel sample introduction method combined to an atmospheric pressure ionization source, the laser diode thermal desorption/atmospheric pressure chemical ionization (LDTD/APCI), coupled to MS/MS. The assembly and schematic diagram of the LDTD/APCI source have previously been reported.<sup>12</sup> The analysis time is achieved in seconds compared to minutes for traditional LC-MS/MS methods by eliminating the LC step. The LDTD/APCI-MS/MS uses a heat gradient to volatilize the analyte of interest which is then ionized in the APCI region upon entering the triple quadrupole system for detection. Our objective in this study is to optimize the method and its operating parameters and identify the limitations. The determination of the selected steroid hormones at low-nanogram per liter levels in effluent wastewater was done to confirm the applicability of the method in real environmental samples, with simple sample preparation, SPE, followed by quantification using the LDTD/APCI-MS/MS system. Method validation was done by evaluating selectivity, linear range, and precision (interday and intraday).

## MATERIALS AND METHODS

**Chemicals, Reagents, and Stock Solutions.** All selected steroid hormones standards (purity  $\geq 97\%$ ), estrone (E1), 17 $\beta$ -estradiol (E2), estriol (E3), 17 $\alpha$ -ethinylestradiol (EE2), levonorgestrel (LEVO), medroxyprogesterone (MPROG), norethindrone (NOR), and progesterone (PROG) were purchased from Sigma-Aldrich (St. Louis, MO). Isotopically labeled estradiol, [<sup>13</sup>C<sub>2</sub>]-E2, used as a surrogate, and 17 $\alpha$ -ethinylestradiol, [<sup>13</sup>C<sub>2</sub>]-EE2, used as an internal standard (IS), were obtained from ACP Chemical Inc. (Montreal, QC, Canada). Individual stock solutions were prepared in methanol (MeOH) at a concentration of 1000 mg/L and kept at  $-20$  °C for a maximum of 3 months. A primary mix of steroid hormone working solution was prepared daily at a concentration of 100 mg/L by dilution in acetonitrile (ACN) of individual stock solution aliquots. Subsequent working solutions were prepared in ACN or water to give solutions of desired concentration. All solvents used were of HPLC grade purity from J. T. Baker (Phillipsburg, NJ), and deionized/distilled water (dd-H<sub>2</sub>O) was used for dilutions.

**Solid-Phase Extraction.** SPE was done using a 12-position manifold manufactured by Phenomenex (Torrance, CA). Reversed-phase Strata-X (surface-modified styrene divinylbenzene polymer) cartridges with a 200 mg bed mass from Phenomenex were used to extract the selected steroid hormones from water matrixes. Prior to dilution (1:10, v/v) in dd-H<sub>2</sub>O and extraction, wastewater effluent samples were collected in precleaned 4 L amber bottles from the St-Eustache wastewater treatment plant (WWTP) (St-Eustache, QC, Canada), filtered using 0.45  $\mu$ m pore size

membranes to eliminate particulate material, and stored at 4 °C to avoid microbial growth. SPE was performed with 500 mL aliquots of the diluted filtered wastewater effluent and dd-H<sub>2</sub>O spiked with an appropriate amount of the mix steroid working solution and surrogate ([<sup>13</sup>C<sub>2</sub>]-E2). The IS ([<sup>13</sup>C<sub>2</sub>]-EE2) was added after the elution step. The SPE cartridges were conditioned with 2  $\times$  3 mL of MeOH followed by 2  $\times$  3 mL of dd-H<sub>2</sub>O. Samples were loaded on the cartridge column at a flow rate of 2–3 mL/min by applying negative pressure using a mechanical pump. After the loading step, the cartridges were air-dried at maximum pressure (7 kPa). The steroid hormones were eluted twice with 3 mL of MeOH into conical-bottom centrifuge tubes. The eluates were evaporated to total dryness under a gentle stream of nitrogen at 40 °C with a nine-port Reacti-vap unit from Pierce (Rockford, IL) and then reconstituted to 300  $\mu$ L with ACN/H<sub>2</sub>O (2:1 v/v) for LDTD/APCI-MS/MS analysis.

**LDTD/APCI-MS/MS.** Ionization of steroid hormones was achieved with the LDTD/APCI ionization source, developed and manufactured by Phytronix Technologies (Quebec, QC, Canada), mounted on a Quantum Ultra AM triple quadrupole mass spectrometer by Thermo Fisher Scientific (Waltham, MA) for analyte detection. Samples were first spotted (1–10  $\mu$ L) into the LazWell 96-well polypropylene plate cavities containing inserts made of proprietary stainless steel alloy with an appropriate solvent and then left to dry at room temperature. The designed well shape allows the sample to concentrate in the heating zone while drying. The loaded plate is then transferred to an X–Y moveable stage of the LDTD housing unit. Upon operation, a glass transfer tube is inserted into a well by an air-powered piston to avoid any sample loss. An infrared (IR) laser diode (980 nm, 20 W, continuous) is then focalized to impact the back of the inserts, thermally desorbing the dried sample, which is vaporized into the gas phase (see the Supporting Information for the LDTD/APCI source principles). The uncharged analyte molecules travel along the transfer tube by a carrier gas (medical grade purified air) to eventually reach the corona region for ionization by APCI and then transferred to the MS inlet.

The LDTD/APCI sample optimization for MS and MS/MS conditions in negative ionization mode (NI) and positive ionization mode (PI) was performed by depositing 2  $\mu$ L of the standard steroid hormone of interest; the IS and surrogate were at a concentration of 2 mg/L in the well plate inserts and are presented in Supporting Information Table S-1. The LDTD/APCI source parameters were set to the following values: corona discharge voltage of 5000 V in PI mode and 5500 V in NI mode, a carrier gas temperature of 50 °C, a sheath gas and auxiliary gas set at 0 for both modes, and the ion transfer tube was set at 350 °C for both modes. Physical parameters of the LDTD/APCI source were optimized to improve signal intensity of spiked aliquots of wastewater effluent in order to account for matrix effects and include solvent choice for analyte deposition, mass of deposition into plate wells, carrier gas flow, laser power, and laser pattern. Analytes were spotted into the sample well once reconstituted in an ACN/H<sub>2</sub>O (2:1, v/v) solution following SPE with a deposition volume of 4  $\mu$ L. Following optimization, carrier gas flow was set at 3 L/min for all selected hormones in both PI and NI mode, except for E3 for which the rate was reduced to 2 L/min,

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while laser pattern programming consisted of a 1 s ramp from 0% to 20%, held for 3 s at 20% before shutting off. The steroid hormones samples were analyzed in three desorption events, i.e., E2, EE2, LEVO, MPROG, NOR, and PROG in PI mode, E1, E2, and EE2 in NI mode, with a different well for E3 which was analyzed separately in NI mode because of the lower gas flow rate (2 L/min) used.

**Data Analysis and Method Validation.** The LD TD/APCI source was controlled by the LazSoft 4.0 software (Phytronix Inc., Quebec, Qc, Canada). Resulting MS/MS peaks were integrated using the ICIS algorithm of the Xcalibur 1.2 software from Thermo Fisher Scientific. A minimum of two selected reaction monitoring transitions (SRM) were used as well as the relative intensities of their ratios so as to avoid false positives and confirm the presence of the detected steroid hormone. In accordance with the European Commission,<sup>13</sup> the SRM ratios were acceptable if for relative intensities greater than 50%, the error was within  $\pm 20\%$  and within  $\pm 50\%$  for relative intensities inferior to 10%. The instrument response was determined as the ratio of the analyte area to that of the isotopically labeled IS.

The recovery values for the SPE method were evaluated at a concentration of environmental relevance, i.e., 50 ng/L, in aliquots of spiked dd-H<sub>2</sub>O containing no wastewater effluent (matrix-free) and in aliquots of the diluted (1:10 v/v) wastewater effluent in dd-H<sub>2</sub>O (in matrix). Extraction recoveries and matrix effects were determined by comparing mean peak areas of the selected steroid hormones spiked prior to extraction in matrix-free samples and matrix-containing samples with those of the selected steroid hormones spiked in postextraction matrix-free samples in triplicates and were reported as percentages. Values below 100% indicate ion suppression, whereas values above 100% represent ion enhancement.

Instrumental limits of detection (ILD) were determined using seven-point, each analyzed in duplicate, internal standard calibration curves in ACN/H<sub>2</sub>O (2:1, v/v) solution free of any effluent wastewater. Method detection limits (MDL) were determined using standard addition calibration curves with four calibration points, analyzed in duplicate, on samples containing diluted effluent wastewater initially spiked at a concentration of approximately 30 ng/L of steroid hormones and an isotopically labeled surrogate before the SPE. The IS was added following the elution step and prior to evaporation at a concentration of 200  $\mu\text{g/L}$ . Three method blank samples (nonspiked wastewater diluted in dd-H<sub>2</sub>O 1:10 v/v) were also added to the SPE procedure. Both ILD and MDL were calculated by multiplying by 3.3 the error on the *y*-intercept and dividing by the slope of the regression line equations.

Interday and intraday precision were determined for a concentration of 100  $\mu\text{g/L}$  of steroid hormones spiked in diluted effluent wastewater following SPE. Intraday precision was calculated as the relative standard deviation (RSD) in percentage of the steroid hormone to IS peak area ratio from then replicates. The interday precision (*n* = 10) was determined by combining the results of this process over 2 consecutive days.

The Statistical Package for Social Science (SPSS 13.0, Chicago, IL) for windows, ANOVA test was used to compare the signal

intensities for the optimization of the LD TD/APCI physical parameters, and we performed the Tukey's *b* test as post hoc test. Statistical significance was defined as a *P* value <0.05.

## RESULTS AND DISCUSSION

**LD TD/APCI Physical Parameters Optimization.** The LD TD/APCI physical parameters must be optimized to improve signal intensity. Aliquots of wastewater effluent were spiked at a concentration of 2 mg/L to account for matrix effects, and samples were analyzed in triplicate.

The LD TD/APCI source produces precursor ions or structure-specific ions upon desorption that are characteristic of the analyte molecules and essential for compound identification in complex matrixes. This is achieved by utilizing rapid sample heating in order to provide sufficient energy to the sample molecules, allowing for vaporization of the intact molecules while minimizing fragmentation. Rapid sample heating is based on a kinetic competition between the temperature dependencies of vaporization (dissociation of intermolecular bonds) and molecular decomposition (dissociation of intramolecular bonds) processes<sup>14</sup> and can be applied to thermally unstable compounds, such as the steroid hormones used in this study, that would normally require a derivatization step or nonvolatile compounds. The heating rate of the LD TD laser is 3000 °C/s which allows the samples to be quickly heated at high temperatures, minimizing the time spent in the decomposition region and favoring vaporization which generates a greater amount of the uncharged molecular species. The actual working temperatures are usually between 100 and 150 °C. Volatilization is also a function of the thickness of the crystal film that forms once the analytes have been deposited into the sample wells and left to dry prior to laser desorption. Previous studies have confirmed that the effective melting temperatures of nanocrystals (whether it is for metals, inert gases, and molecular crystals) can be significantly lowered in relation to their decreasing size compared to the bulk melting point.<sup>15,16</sup> This phenomenon could be related to the adequate dispersal of nanocrystals onto the plate well surface upon drying, which would reduce surface–molecule interactions due to poor affinity between the stainless steel alloy well surface and the analytes as well as molecule–molecule interactions compared to bulk material. Consequently, the temperature needed to overcome the bonding energies and allow for volatilization is below the bulk melting point. This suggests that, for a given product, if decomposition is favored before melting can occur in the bulk state, it can still be analyzed using the LD TD/APCI source. Therefore, optimization of the type of solvent in combination with the energy (laser power) needed to vaporize our compound is done simultaneously.

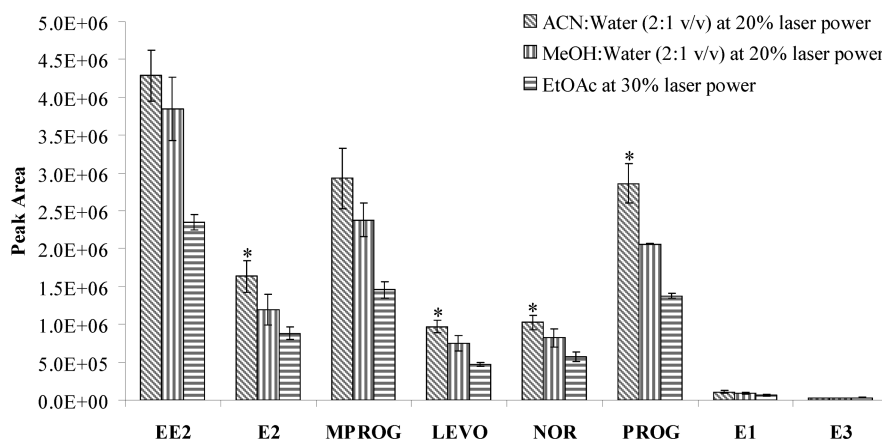
The solvent used for analyte deposition should have a surface tension superior to 27 mN/m to prevent the sample droplet to flow outside the designed cavity of the sample well. Figure 1 compares the peak intensity of the eight selected steroid hormones for three different solvents used for analyte deposition at

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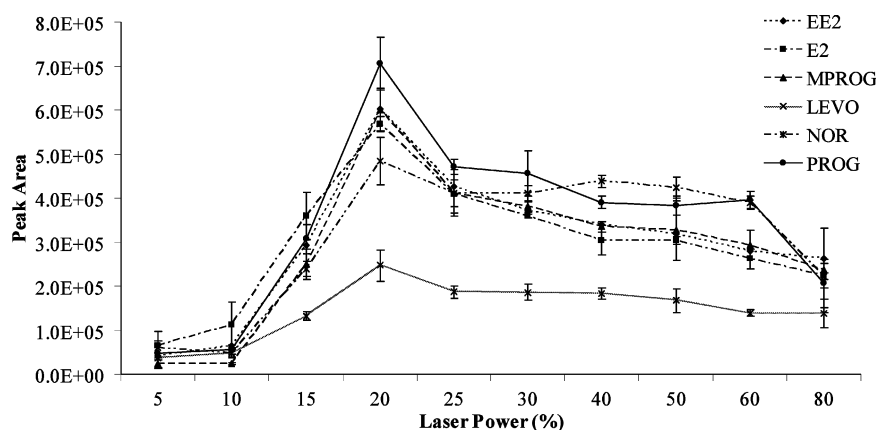
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**Figure 1.** LDTD/APCI optimization of three different solvent solutions with their corresponding respective optimum laser power, ACN/H<sub>2</sub>O (2:1, v/v) and MeOH/H<sub>2</sub>O (2:1, v/v), both at 20% laser power, and pure EtOAc at 30% laser power, used for analyte deposition in plate well. The concentration used was 2 mg/L. ACN/H<sub>2</sub>O (2:1, v/v) was determined to be the solvent of choice with four out of the eight hormone steroids having a significantly higher ( $n = 3$ ;  $P < 0.5$ ) peak area intensity represented by a star symbol (\*) than with MeOH/H<sub>2</sub>O (2:1 v/v) or pure EtOAc.



**Figure 2.** Positive ionization mode (PI) maximum peak intensity for a concentration of 2 mg/L was observed at a maximum 20% laser power for all selected steroid hormones and was significantly different from other laser power ( $n = 3$ ;  $P < 0.5$ ).

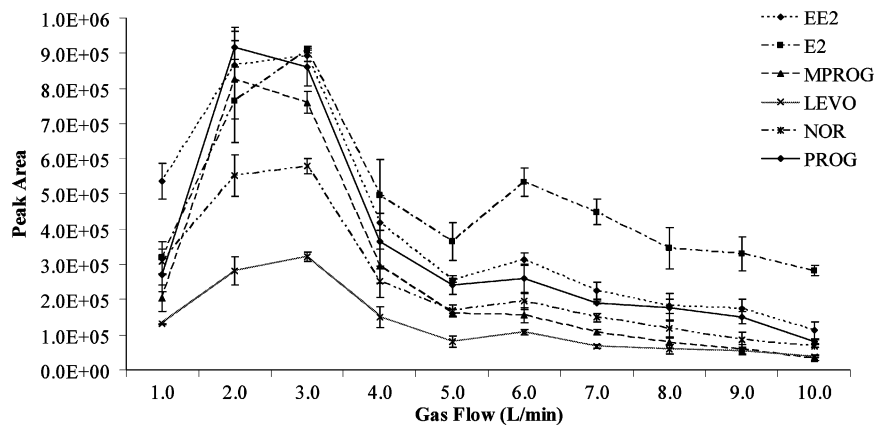
their respective optimal laser desorption setting, i.e., ACN/H<sub>2</sub>O (2:1, v/v) at 20% laser power, MeOH/H<sub>2</sub>O (2:1, v/v) at 20% laser power, and pure ethyl acetate (EtOAc) at 30% laser power. The ACN/H<sub>2</sub>O (2:1, v/v) solution gave significantly higher ( $P < 0.05$ ) peak area intensities with RSD under 15%, for four out of the eight selected steroid hormones and therefore was the preferred solution used for analyte deposition into the well cavity for the rest of the experiments. The optimal laser power was determined to be 20% (Figure 2 and Supporting Information Figure S-2) for ACN/H<sub>2</sub>O (2:1, v/v) as a deposition solvent in both PI and NI mode according to statistically higher ( $P < 0.05$ ) peak intensity associated with low signal variability (RSD < 15%). The only exceptions were EE2 and E1 in NI mode (Supporting Information Figure S-2) where both 15% and 20% were statistically alike, but 20% was chosen to have a single method for all steroid hormones in NI mode. Working above the optimal laser power resulted in ion suppression by thermal fragmentation of the neutral species due to elevated desorption temperature, whereas at under 20% the desorption temperature was not sufficient to completely volatilize the steroid hormones.

Carrier gas flow was optimal between 2 and 3 L/min (Figure 3 and Supporting Information Figure S-3) and gave significantly higher peak area response ( $P < 0.05$ ) in both PI and NI mode.

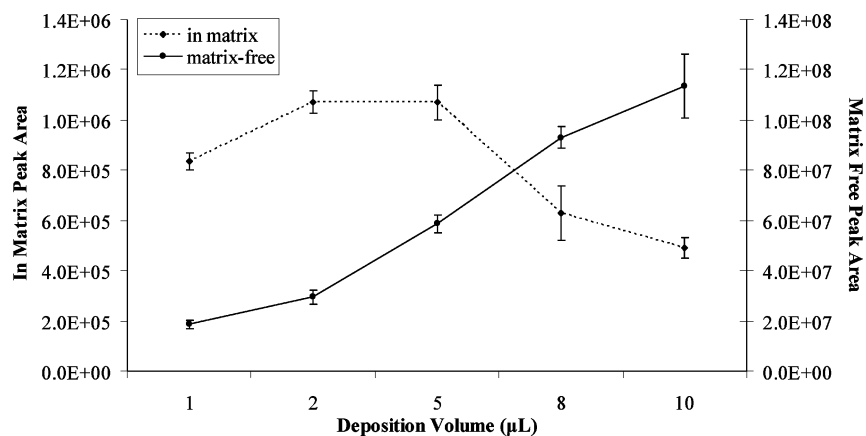
However, a gas flow of 3 L/min provided the best signal-to-noise ratio combined with small signal variability (RSD < 10%) than to 2 L/min. The only exception was E3 in NI (Supporting Information Figure S-3), a more polar steroid than the others, for which we used a gas flow of 2 L/min.

The deposition volume will influence the amount of material loaded into the sample well and could affect the effectiveness of the APCI by either ionic suppression by proton affinity competitiveness or by trapping the compounds into nonvolatile matrix products, thus limiting the signal intensity. Indeed, one way of increasing sensitivity and improve the MDL is to enhance the signal by adding more sample, therefore more analyte, into the sample well cavities. Figure 4 demonstrates the effect of deposition volume on peak area intensities for PROG in PI mode and illustrates ionic suppression as seen for the remaining selected steroid hormones. As expected, the peak areas of PROG progressively increase with increasing deposition volume when no matrix (effluent wastewater) is added to the samples, whereas a lower peak area response is observed for a deposition volume superior to 5  $\mu$ L when matrix is introduced with the sample for a given concentration.

The laser pattern programming that gave the maximum peak area intensity with less variability (RSD < 10%) consisted of a 1 s



**Figure 3.** Carrier gas flow was optimal between 2 and 3 L/min and gave significantly higher peak area response ( $n = 3$ ;  $P < 0.5$ ) in PI mode. A gas flow of 3 L/min provided the best signal-to-noise ratio combined with small signal variability (RSD < 10%;  $n = 3$ ).



**Figure 4.** Effect of deposition volume change on method sensitivity and matrix-induced peak area intensity in spiked effluent wastewater compared to intensities in matrix-free solvent (ACN/H<sub>2</sub>O (2:1, v/v)) for PROG in PI mode. In matrix-free solvent, the signal for PROG increases with an increase in deposition volume, whereas in spiked effluent wastewater matrix, the peak area intensity diminishes significantly for deposition volumes greater than 5  $\mu$ L at an identical concentration of 2 mg/L.

ramp from 0% to 20%, held for 3 s at 20% before shutting off the laser (Supporting Information Figure S-4). Increasing holding time past 3 s, up to 9 s, at 20% with the same laser pattern did not improve peak area intensities (results not shown) and would have lengthened the analysis time.

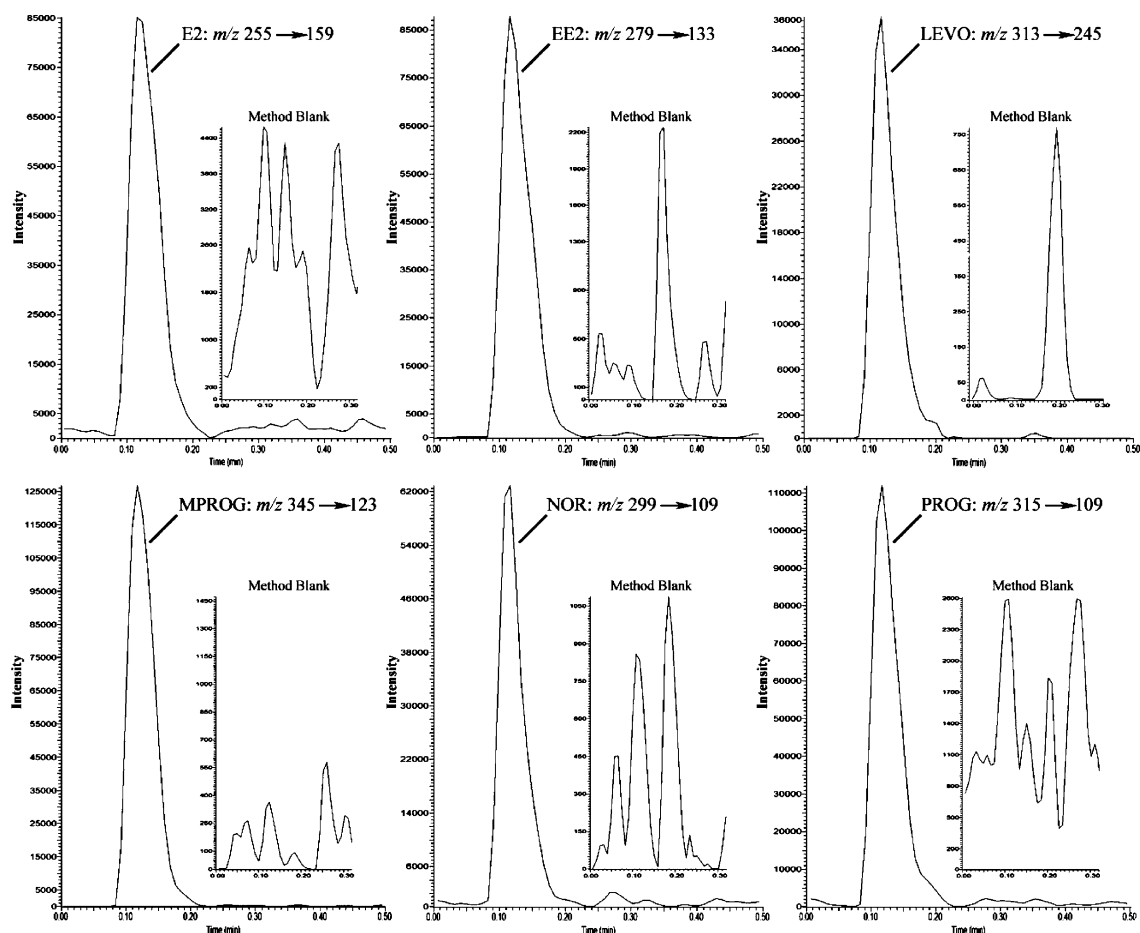
**Method Application and Validation.** The LDTD/APCI-MS/MS method with the described optimized physical parameters discussed previously was applied to spiked diluted effluent wastewater at an environmentally relevant concentration of 30 ng/L. The analysis took 15 s per desorption event, with three desorption events per sample. For a given sample, three plate wells were desorbed, with one well analyzed for six compounds (E2, EE2, LEVO, MPROG, NOR, and PROG) in PI mode (Figure 5), another for three compounds (E1, E2, and EE2) in NI mode, and a third well for the analysis of E3 in NI mode. The third well was analyzed because a lower gas flow rate (2 L/min) was optimal for E3. The total analysis time from well to well was 40 s.

The samples were concentrated using SPE and gave good extraction recoveries for a concentration of 50 ng/L (Supporting Information Figure S-5) ranging from 84% to 111% in matrix-free (pure water) samples and from 77% to 121% in spiked effluent wastewater (in matrix) samples for all selected steroid hormones with RSD inferior to 20% in all cases.

Adequate calibration curves were produced with good linearity (Table 1) even for low concentrations in matrix-free solvent (internal calibration) with a linearity range of 9–915  $\mu$ g/L and in spiked effluent wastewater (standard addition calibration) with each calibration point analyzed in duplicate and the unknown in triplicate. The coefficients of determination ( $R^2$ ) were excellent with values ranging from 0.9986 to 0.9999 for internal calibration in matrix-free samples and from 0.9950 to 0.9997 for standard addition for sample containing wastewater effluent. Instrumental limits of detection and method detection limits calculated from the calibration curves were from 6 to 24  $\mu$ g/L and 13 to 42 ng/L, respectively (Table 1). The resulting MDL are comparable to several other analytical methods applied to steroid hormones, including online SPE coupled to LC-MS/MS<sup>17,18</sup> and off-line SPE methods using GC/MS.<sup>19</sup>

Precision measurements were acquired for each compound and were satisfactory (Table 2). The intraday precision was better than 14% for all the analytes ( $n = 10$ ), whereas the interday precision was better than 15% for all the analytes except for EE2

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**Figure 5.** MS/MS spectra of the product ions (SRM) of one desorption event for the lowest concentration calibration point (wastewater, diluted 1:10 v/v in dd-H<sub>2</sub>O and spiked at 30 ng/L) used in the standard addition calibration curves for E2, EE2, LEVO, NOR, MPROG, and PROG in PI mode. The method blank mass spectra (inset), representing nonspiked diluted wastewater (1:10 v/v) samples, are also shown to confirm the absence of any peak contribution from nonspiked wastewater and to evaluate the average background noise.

**Table 1. Linearity (Coefficient of Determination), Sensitivity, Instrumental Limits of Detection (IDL), and Method Detection Limits (MDL) of the LDTD/APCI-MS/MS Method for the Selected Steroid Hormones in Negative (NI) and Positive (PI) Ionization Modes**

compound	ionization mode	matrix-free				in matrix		
		linearity range ( $\mu\text{g/L}$ )	sensitivity	$R^2$	ILD ( $\mu\text{g/L}$ )	sensitivity	$R^2$	MDL (ng/L)
E1	NI	9–684	0.0421	0.9999	9	0.0129	0.9984	30
E2	NI	18–588	0.0084	0.9998	10	0.0030	0.9972	36
	PI	18–588	0.0384	0.9995	5	0.0035	0.9997	13
E3	NI	80–749	0.0038	0.9986	24	0.0016	0.9977	30
EE2	NI	39–915	0.0062	0.9993	17	0.0019	0.9946	42
	PI	57–915	0.0178	0.9990	8	0.0024	0.9988	14
LEVO	PI	47–780	0.0128	0.9990	11	0.0025	0.9965	20
MPROG	PI	35–677	0.0630	0.9990	6	0.0098	0.9964	30
NOR	PI	41–634	0.0144	0.9989	9	0.0031	0.9950	25
PROG	PI	21–670	0.0320	0.9995	6	0.0080	0.9971	18

in NI mode which was at 17% ( $n = 10$ ). The impact of cross-contribution from the isotopic patterns of steroid hormones with a two mass unit difference, i.e., E2 with E1 in NI mode, LEVO with PROG in PI mode, and E2 or EE2 with their isotopically labeled counterparts (surrogate and IS), was considered insignificant (less than 5% in all cases) and did not affect accuracy (% bias, Table 2).

Method applicability to concentrations naturally found in environmental matrixes has been demonstrated. The complete

procedure, SPE followed by LDTD/APCI-MS/MS, was tested with success (Table 2) to the spiked wastewater effluents aliquots at 30 ng L<sup>-1</sup> with bias under 20% for all selected steroid hormones except for E1 in NI mode and LEVO in PI mode at 21% and 23%, respectively. Nonspiked wastewater effluent aliquots ( $n = 3$ ) were also analyzed as method blanks (Figure 5) to confirm the absence of any peak contribution from nonspiked wastewater diluted 1:10 v/v in dd-H<sub>2</sub>O.

**Table 2. LD TD/APCI-MS/MS Analysis of Known Amounts of Selected Steroid Hormones Spiked Diluted Wastewater Effluent Samples with Associated Bias Values along with Method Intraday and Interday Precision in Negative (NI) and Positive Ionization Modes<sup>a</sup>**

compound	ionization mode	method application in matrix			precision	
		amount added (ng/L)	amount found (ng/L)	bias (%)	intraday RSD (%)	interday RSD (%)
E1	NI	31.5	38 ± 4	21	9	14
E2	NI	33.5	37 ± 2	9	13	15
	PI	33.5	31 ± 2	7	6	7
E3	NI	34.2	35 ± 4	1	9	14
EE2	NI	33.9	39 ± 8	15	14	17
	PI	33.9	29 ± 2	15	4	5
EE2-13C2	NI	30.0	35 ± 5	17	n.a	n.a
	PI	30.0	28 ± 2	8	n.a	n.a
LEVO	PI	38.5	30 ± 2	23	9	13
MPROG	PI	32.4	27 ± 2	17	7	10
NOR	PI	35.3	28 ± 3	20	5	15
PROG	PI	32.2	30 ± 3	7	7	10

<sup>a</sup> Means values of triplicate analyses are given with standard deviations for the amounts found.

## CONCLUSION

We have demonstrated the different optimization and operation parameters for the LD TD/APCI-MS/MS, a simple, sensitive, rapid, and reliable method which allows for high-throughput sample analysis and quicker data generation. These parameters included optimal solvent used for analyte deposition into the sample well cavities determined to be a solution of ACN/H<sub>2</sub>O (2:1, v/v), desorption laser power which was set at 20%, and carrier gas flow set from 2 to 3 L/min, and maximum deposition volume

(5 μL) which will be dependent on matrix complexity and cleanup procedures. The determination of eight steroid hormones, spiked at 30 ng/L in diluted effluent wastewater, was done to confirm the applicability of the method in real environmental samples, with simple sample preparation (SPE using Strata-X) with recoveries ranging from 77% to 112% with RSD under 20%, followed by quantification using the LD TD/APCI-MS/MS system. Calibration curves were linear, with  $R^2 > 0.99$  for all steroid hormones in both PI and NI mode, and the resulting precision variation (intraday and interday) was less than 20%. Method detection limits ranged from 13 to 42 ng/L and were in agreement with several published methods on steroid hormones. The analysis time is achieved in only 40 s from sample to sample compared to minutes for traditional LC-MS/MS methods by eliminating the chromatography step and therefore also reducing the cost and environmental impact related to column and solvent consumption.

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## SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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