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Rapid on-line extraction and quantification of escitalopram from urine using sol–gel columns and mass spectrometric detection

Nina Johannesson, Jonas Bergquist *

Analytical Chemistry, Department of Physical and Analytical Chemistry, Biomedical Centre, Uppsala University, Box 599, SE-751 24 Uppsala, Sweden

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Abstract

A fast and easy way to quantify escitalopram in urine has been developed. A capillary with a silica based monolithic bed inside is used to extract escitalopram from urine and the for mass spectrometry detrimental matrix is washed away by applied pressure. The analyte is eluted by a solution containing organic modifier and directly electrosprayed into a time-of-flight mass spectrometer, ESI-TOF-MS. This method makes it possible to load large volumes of sample onto the column and preconcentrate escitalopram on-line before detection. Standard addition of escitalopram to the urine sample gave a linear calibration curve ($R^2 = 0.988$). The analyzed sample was found to contain an escitalopram concentration of 0.62 ng/ml, well in line with earlier publications. The calculated LOD was 10 pg/ml and LOQ was 34 pg/ml as compared to earlier reports with a LLOQ of 1 ng/ml. The intra day variation of the escitalopram peak area is less than 6.3%.

Keywords: Escitalopram; Urine; Sol–gel; ESI-TOF-MS; On-line; Sample cleanup

1. Introduction

Depression is a growing problem in today's society and numerous neuroleptic drugs have been developed to mitigate this problem. Escitalopram is the S-enantiomer of racemic citalopram which is a highly selective serotonin re-uptake antidepressant [1,2]. The half-life in the human body of escitalopram is 27–32 h and the detection of the drug can be desirable in toxicology and forensics [3,4]. Finding drugs in biological samples are usually a difficult task since the matrix is often very complex. In urine, for example, high concentrations of urea and salts complicate the detection. Pretreatment of the samples as centrifugation, precipitation, solvent extraction, liquid–liquid extraction (LLE), solid-phase extraction (SPE) and ultrafiltration [5] are routine. The standard procedure for sample cleanup before the detection of antidepressant is liquid–liquid extraction after alkanization with potassium borate, sodium carbonate, sodium hydroxide or potassium hydroxide. A variety of organic solvents as hexane-isoamylalcohol, *n*-butyl chloride, diethyl ether or *n*-heptane-ethylacetate are used [3]. Occasionally, some acidic back extraction is acquired before separation [6–8]. SPE cleanup is also frequently used but the recoveries can very quite a lot [9]. Most of these techniques are performed off line, are time consuming and increase the risk of losing the analyte of interest.

Most often, serum is used during antidepressant analyses since the drug concentration is approximately 90% higher in serum than in urine [10] but the sampling is also more complicated and a more sensitive method for urine analyses is desirable. High performance liquid chromatography and gas chromatography are standard separation methods for drug analysis and they both require sample pretreatment to perform accurate analyses of body fluids. The combination of these existing sample cleanup and separation techniques result in LOD and LOQ in the area of 1–5 and 25 ng/ml, respectively, which is not sufficient for urine analysis.

In recent years, a trend of integrated preconcentration and extraction techniques has been growing. Particles used in liquid chromatography have been utilized [11–16] but the frits holding them in place often giving rise to bubble formation in electrophoresis [17–20]. Alternative methods are therefore desirable and alternatives as to bind the particles together with sol–gel

* Corresponding author. Tel.: +46 18 471 3675; fax: +46 18 471 3692.
E-mail address: jonas.bergquist@kemi.uu.se (J. Bergquist).

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technology [21] or to have a wall coating [22–25], monolithic bed [26–29] or a membrane [30–33] inside the column have been reported.

Solid-phase preconcentration with a packed segment produces quite a lot of back pressure and the CE performance may be compromised [11,12,16]. With an open tubular concentrator the phase ratios are rather limiting and the presence of a membrane requires coupling of different segments.

Monolithic columns generate less back pressure and all sorts of modifications can be used like incorporation of proteins to develop highly selective phases [34,35].

In this paper, a fast and easy way of sample cleanup on-line is presented. As described earlier [36], a sol–gel monolith inside of a fused-silica capillary works as a solid-phase extraction bed that adsorb hydrophobic species from a complex biological sample. No valves or couplings are needed in this setup. The column is directly connected to a mass spectrometer (MS) for sensitive and selective detection. The untreated sample is directly loaded onto this column and the matrix is washed away before the escitalopram is eluted for detection. The simple setup makes the total analysis time very rapid and no sample is lost during the procedure.

2. Experimental

2.1. Chemicals and reagents

The reagent chemical 3-(trimethoxysilyl)propyl methacrylate, 99% was purchased from Sigma (St. Louis, MO, USA), Irgacure 1800 from Ciba (Tarrytown, NY, USA) and ethanol from Solveco, Chemicals AB (Täby, Sweden). Solvents and acids were purchased from MERCK (Darmstadt, Germany) and the water was filtered using a Milli-Q$^+$ system (Millipore Corp., Marlborough, MA, USA). The octadecyltrimethoxysilane, 90% for modification came from Sigma-Aldrich (Milwaukee, WI, USA). Fused-silica capillaries (75-μm i.d. × 360-μm o.d.) were purchased from Polymicro Technologies (Phoenix, AZ, USA). Escitalopram was obtained by dissolving Cipralex® (15 mg) (Lundbeck, Copenhagen, Denmark) in Milli-Q water.

2.2. Instrumental setup

A capillary electrophoresis (CE) instrument from Hewlett-Packard, Germany, ^{3D}CE, was coupled to a mass spectrometer, MS. The MS used in these experiments was a LC/MSD TOF from Agilent Technologies, USA. The sheath liquid consisted of 50/50 (v/v) methanol/water with 5 mM acetic acid and was introduced at a flow rate of approximately 2 μl min^{-1}. A voltage of approximately 4.0 kV was applied to induce electrospray.

2.3. Column preparation

To prepare the sol–gel solution, 575 μl of 3-(trimethoxysilyl)propyl methacrylate and 100 μl of 0.12 M hydrochloric acid were mixed and stirred for 30 min at room temperature in the dark. Of that mixture, 120 μl was added to 480 μl of toluene (porogenic agent) and 60 mg of Irgacure 1800 and stirred with a magnetic bar for 5 min at room temperature in the dark. A 5-cm long exposure window for UV polymerization was created on a fused-silica capillary (75-μm i.d.; 360-μm o.d.) by removing a narrow strip of the polyimide coating. The removal procedure is accomplished with a razor blade as earlier described by Dulay et al. [37]. The capillary was then filled with the sol–gel solution and plugged with parafilm (Parafilm, American National Can™, USA) at both ends before being irradiated at 365 nm for 4.5 min in a crosslinking apparatus (BIO-LINK® BLX, Marne-la-Vallée, France). To remove unpolymerized solution, the column was flushed with ethanol using a syringe pump (CMA/102, CMA/Microdialysis AB, Sweden) for about 30 min. The total length of the column was 55 cm and the 5-cm sol–gel section was located approximately 13 cm from the injection end.

2.3.1. C18 modification

Derivatization with the C18 silane reagent was performed by flushing the column with neat octadecyltrimethoxysilane, $CH_3(CH_2)_{17}Si(OCH_3)_3$, solution for 60 min (approximately 2 μl min^{-1}) at 20 °C and then rinsing with ethanol for 30 min.

2.4. Sample and separation solutions

The running buffer consisted of 5 mM ammonium acetate (pH 6.8) in 30% water and 70% acetonitrile. To rinse the column after urine injection, a washing buffer of 5 mM ammonium acetate, pH 6.8, in water was used. The urine was collected from a female volunteer medicated with Cipralex®, 15 mg/day and filtered using a Schleicher & Schuell (Dassel, Germany), FP 030/8, 0.2-μm pore-size filter, and stored at −20 °C. The standard solution of escitalopram was made by dissolving one tablet of Cipralex® (15 mg) in 15 ml milli-Q water (30 min in sonicator) and then centrifuged at 2000 rpm for 5 min + 2500 rpm for another 5 min in room temperature. The supernatant was transferred to clean tubes and stored at −80 °C until use. Thawed samples were kept at 6 °C and found to be stable for up to 30 days.

3. Results and discussion

3.1. Quantification of escitalopram in urine

To quantify the amount of escitalopram in urine a standard addition curve was created since no suitable internal standard was identified. The escitalopram concentration in the sample showed to be over the sample loading capacity for the column why the urine was diluted 10 times in Milli-Q water. Three additions of escitalopram were done to the diluted urine, as described in Table 1. The sample was injected onto the column for 3 min (1.35 μl) and to wash away the matrix a rinsing solution was flushed through the column for 15 min (4 column volumes or 6.8 μl). To elute the analytes, the injection end of the column was placed in the separation solution consisting of 5 mM ammonium acetate and 70% acetonitrile. A potential of 20 kV was applied

Table 1
Standard addition of escitalopram to urine sample

Sample number	Urine (ml)	Added amount escitalopram (ng)
1	19.50	–
2	20.29	9.47
3	19.69	27.57
4	19.86	45.30

and the ion traces of urea and escitalopram is shown in Fig. 1. A linear regression with equation $y = 8767.2x + 98685$ and a good linearity ($R^2 = 0.9881$) was obtained for the standard addition curve and the calculated concentration of escitalopram in the urine sample was found to be 0.62 ± 0.3 ng/ml urine. The concentration is slightly lower than reported by Sogaard et al. [10] who reported a concentration of approximately 1.6 ng/ml but they also stated that the body weight and food intake influences the drug level. The sample is reported to be stable over time by Singh et al. [38] why no degradation should have occurred. The rate of the human metabolism is very individual so the level of escitalopram in urine could vary a lot. The sample was collected during the day without any restriction in water consumption, why the urine may well be somewhat diluted. It would have been possible to correlate the obtained concentration of escitalopram with, i.e. creatinine level, etc., but this was not the scope of this paper. Also a robust internal standard would be advantageous to allow for compensation of both variations in sample cleanup and electrospray fluctuations.

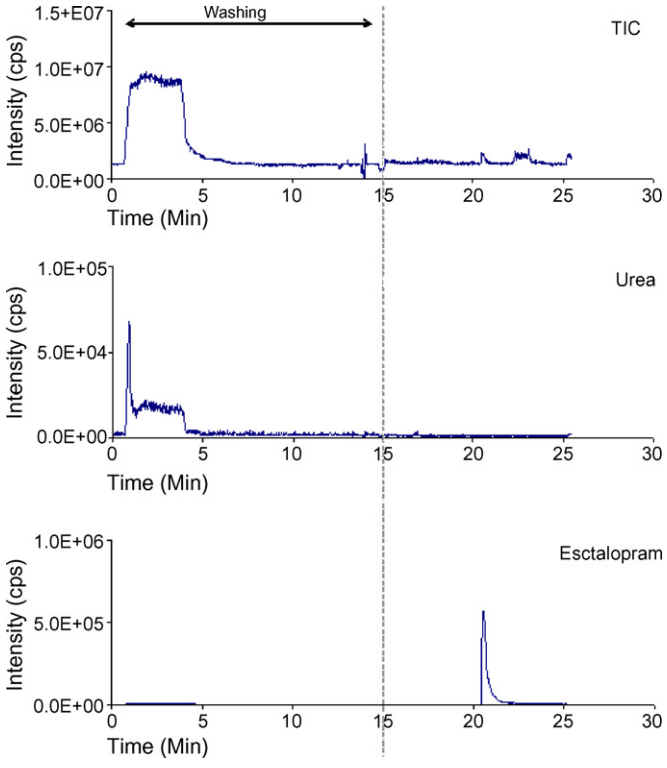

Fig. 1. Total ion count and ion traces of urea (61 *m/z*) and escitalopram (325 *m/z*) during cleanup with pressure (15 min) and elution with high voltage, 20 kV (15–25 min).

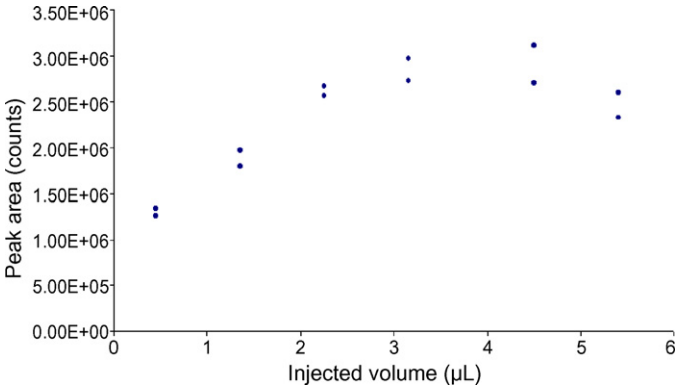

Fig. 2. Loading capacity of escitalopram on C18 modified sol–gel column.

3.2. Loading capacity

To evaluate the loading capacity of the PSG, different volumes of urine sample (diluted 10 times) were loaded onto the column. Since there are many other natural occurring hydrophobic species in urine, besides escitalopram, more molecules should be competing for the extraction sites in larger injection volumes. Fig. 2 shows the peak area of escitalopram for different injection volumes. The system seems to handle volumes up to 2.25 μl, which is ~1.4 column volumes after which the signal saturates. This limit is probably due to saturation of the extraction sites since the obtained limit is in line with loading limits of urine reported earlier [36], but there might also be a risk of ion suppression in the ESI process.

3.3. Limit of detection

From the smallest volume injected (0.45 μl) the limit of detection, LOD (3 × S/N), and limit of quantification, LOQ (10 × S/N), was calculated. The injected amount of escitalopram was 0.28 pg and if the relation between concentration and signal is assumed to be linear, the LOD is 23 fg and the LOQ is 77 fg. Since the maximum loading capacity of the system is estimated to approximately 2.25 μl the concentration of LOD and LOQ are 10 and 34 pg/ml, respectively.

3.4. Repeatability

The variation within day in peak area is less than 6.3% and the majority of that variation is known to be a result from the ESI process. Since no internal standard is used in this study we could not compensate for this source of error.

4. Conclusions

The present method has shown to be a fast and easy way to determine the escitalopram concentration in urine. Large volumes of sample (in CE measures) can be loaded onto the column and directly analyzed by MS. No sample pretreatment is acquired except for filtration and dilution of the sample. Compared to earlier methods of escitalopram quantification this method is time saving and offer lower detection limits since no

apparent sample loss is occurring along the way. The procedure of column making is easy and well established by several groups. A suitable internal standard would make the method very reliable for quantification by ESI-MS.

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