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Rotating multitip micropillar array electrospray ionization-mass spectrometry for rapid analysis and high-throughput screening

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ABSTRACT

The applications of rapid screening benzodiazepines from urine and determining synthetic reaction products and kinetics semi-quantitatively using a microfabricated rotating multitip electrospray ionization (ESI) platform for mass spectrometry (MS) is presented. The ESI tips are based on lidless micropillar array ESI (μPESI) sources where the transfer of liquid is based on capillary forces without external pumping. A single silicon platform contains 60 separate μPESI tips which can be individually used by rotating the whole platform by six degrees each time from tip to tip using a computer control. The rotating multitip μPESI platform with an ion trap mass spectrometer was successfully demonstrated in rapid identification and monitoring of intermediates and final products in chemical synthesis within 10 min. The system was also applied to high-throughput screening of benzodiazepines from urine samples. Urine samples were extracted with solid-phase extraction (SPE) using C_{18} phase ZipTip™ pipettes, enabling the use as small sample volumes as 50 μL of the urine sample. Therefore the whole sample treatment and the analysis with the rotating multitip μPESI-MS took only 5 min per sample.

1. Introduction

A current trend in analytical chemistry has been miniaturization and microfabrication of analytical instruments and integration of different kind of functionalities on the same microchip. One important research field in analytical chemistry has been miniaturization of ion sources for mass spectrometry [1,2]. For example, various miniaturized electrospray ionization (ESI) sources have been developed from glass [3,4], silicon [5], and different types of polymers [6,7]. The miniaturized ion sources can increase ionization efficiency and thereby the sensitivity of measurements, and minimize the use of organic solvents [8]. Due to low manufacturing costs, the microchip ion sources can be replaced, when their performance is not any more acceptable in terms of sensitivity and stability, or they can be disposable if needed.

To speed up analysis cycle time and to diminish contamination risks, multi ion sources have been recently developed, Nanomate™ being the most common of them [9]. The Nanomate system contains an array of nanoelectrospray needles etched in a silicon wafer. Typical flow rates used in systems range from 20 to 300 nL/min. It has three operation modes, namely chip-based infusion, LC–MS fraction collection, and liquid extraction surface analysis (LESA™) [10]. These modes can be used for example, in rapid analysis of proteins [11] and lipids [12], drug and metabolite analysis [13,14], and surface analysis of tissues [15]. Other multi-array ion source systems are, for example, a multichannel device with an array of ESI tips [16] chemically etched fused-silica ESI emitter for improved sensitivity [17], an array of enclosed ESI tips made from an SU-8 polymer [18], and a micromachined silicon-based ultrasonic ejector array [19]. A rotating ion source platform has been previously designed, using a polycarbonate microdevice with radial closed channels extending to the circumference of the disk where an opening at the rim acted as an ESI tip [20]. Another microfabricated ion source platform suitable for automation, a rotating sample surface for desorption electrospray ionization MS (DESI/MS), was previously published [21]. In this system suitable surface spots for DESI were printed on the edge of a CD disc (totally 50 spots), and after introduction of a sample to the sampling spot, DESI/MS was applied to a rapid analysis of the sample. In this way a rapid quantitative determination of caffeine in two diet sport

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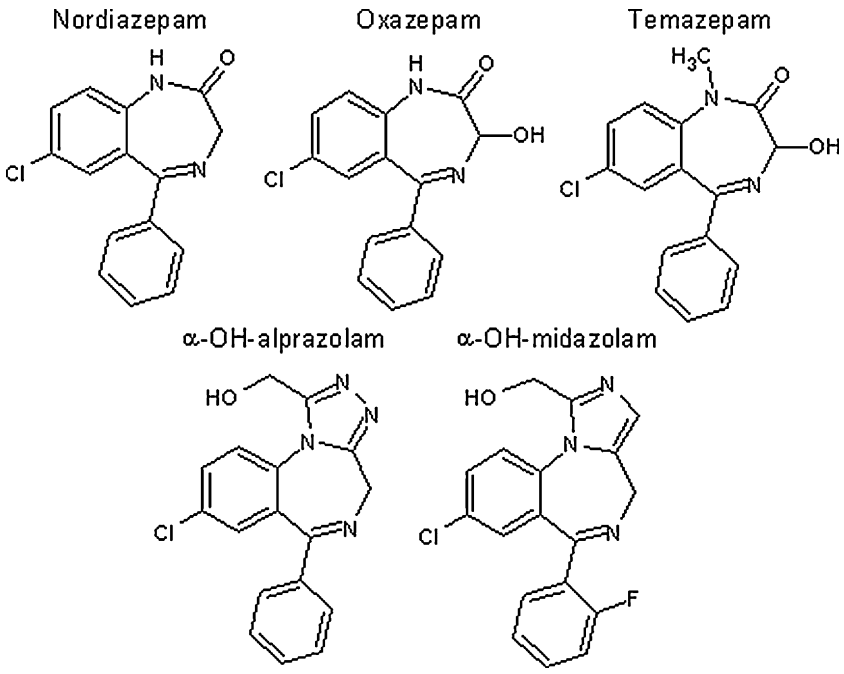

Fig. 1. Chemical structures of the benzodiazepines studied.

drinks using isotopically labeled caffeine as an internal standard was demonstrated. The total analysis time of seven calibration standards and two sport drink samples (45 separate analyses) was 7.5 min. A similar rotating multinozzle emitter array for nanoelectrospray ionization was recently fabricated from silicon [22]. The platform consisted of 96 identical 10-nozzle emitters in a circular array on a 3-in. silicon chip. For sampling the inlets on the silicon platform were connected to 96 silica capillaries via the PTFE tubing, thus enabling high-throughput measurements.

We have previously shown that an open silicon micropillar array channel can induce a spontaneous sample transfer to a sharp tip where an electrospray plume is formed [23,24]. The open channel enables very easy sampling of liquid samples onto the microchip and a rapid transfer of the liquid to the tip of the microchip for ESI. The open micropillar array channel made of SU-8 polymer can also be used as a microreactor for protein digestion studies [25]. When a silicon based μPESI microchip was covered with titanium dioxide (TiO_2) it could be used for performing of photocatalytic reactions and mimicking phase I metabolism with an UV lamp, and a direct analysis of reaction products with MS [26]. The μPESI chip has also been covered with a glass lid to enable liquid chromatographic separation using the micropillar array as a stationary phase combined with a monolithically integrated μPESI tip for MS detection [27]. The μPESI system has also been fabricated in a rotating multitip format [28]. In this format a silicon wafer has 60 individual ESI tips at the periphery of a circular microchip platform. It was shown that a computer-controlled turning from one tip to another one improves repeatability from tip-to-tip because positioning the microchip in front of the mass spectrometer was much easier and more repeatable. In addition, the analysis times were shorter and contamination was minimized as an unused tip was possible to use with every single sample.

The aim of the study was to evaluate the performance of the rotating multitip μPESI source in rapid analyses. The feasibility of the multitip-μPESI was studied in the rapid follow-up analysis of synthesis processes and in high-throughput screening analysis of abused drugs in urine samples using a quick sample preparation method of ZipTip™ solid-phase extraction (SPE) [29,30] prior to the measurement with μPESI/MS. The multitip μPESI platform was combined to ion trap and time-of-flight (TOF) mass spectrometers.

2. Experimental

2.1. Chemicals and samples

HPLC-grade acetonitrile, methanol, and formic acid were obtained from Sigma (St. Louis, Mo, USA). Water was purified with Milli-Q purification system (Millipore, Molsheim, France). A solvent used for flushing the microchip before measurements consisted of 95% acetonitrile or methanol and 1% formic acid in water. 1 mL of 100 μM samples of benzodiazepines in methanol, namely nordiazepam, oxazepam, temazepam, α-OH-alprazolam, and α-OH-midazolam were obtained from United Medix Laboratories Ltd. (Helsinki, Finland). The structures of the compounds are shown in Fig. 1. Pooled urine and authentic positive urine samples, analyzed previously with immunological and GC–MS methods, were obtained from United Medix Laboratories Ltd. Spiked urine samples were prepared to contain all benzodiazepines with a cut-off concentration of 200 ng/mL in pooled urine.

2.2. The rotating multitip μPESI microchip

The basic analytical properties and fabrication process of the rotating multitip μPESI microchips have been previously published [28]. Briefly, the 60 tip microchips are fabricated on a silicon wafer. The fabrication process uses two mask levels: nested masks of silicon dioxide (SiO_2) and aluminum (Al). The SiO_2 mask defines the microreactor chamber and the fluidic channel embedded with micropillars while the aluminum mask defines the sharp thru-wafer etched ESI tip. The ESI tip, the sampling spot, and the flow channel are created with deep reactive ion etching (DRIE). Both the microreactor and the flow channel contain micropillars (diameter 60 μm, space between the pillars 15 μm, height 22 μm, Fig. 2c) to support spontaneous sample flow towards the electrospray tip. There are 60 tips on one platform and the angles between the tips are 6 degrees (Fig. 2a and b).

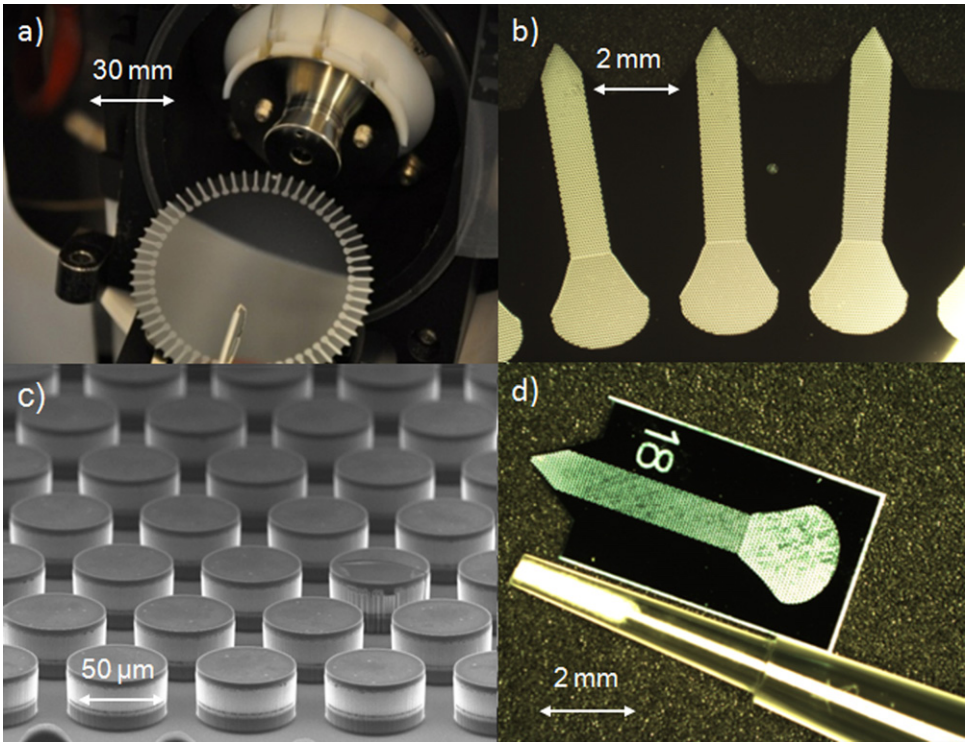

Fig. 2. (a) A rotating multitip-µPESI platform containing 60 individual tips combined with MS. (b) Three individual µPESI tips on a rotating multitip platform. Width of the pillar array channel is 1 mm. (c) A scanning electron micrograph of a micropillar array of the chip. (d) A single µPESI chip with a ZipTip solid-phase extraction pipette.

2.3. Mass spectrometry

Waters Q-TOF Micro (Waters, UK), Bruker MicroTOF (Bruker Daltonics, Germany), and Agilent 6330 Ion trap (Agilent Technologies, Santa Clara, CA, US) were used in MS experiments. The Q-TOF and MicroTOF, which were used in the drug screening, were calibrated using a sodium formate solution in external calibration. The microchip positioned in front of the cone of a mass spectrometer to the distance of 5 mm (Fig. 2a). Nitrogen produced by a high-purity nitrogen generator was used as a cone gas (flow 40 L/h, cone temperature 80 °C). A platinum electrode connected the high voltage source of the MS to the microchip. The high voltage of 3.5 kV was used in experiments for electrospray ionization in a positive ion mode. A mass range of *m/z* 80 to 800 was monitored. With Agilent 6330 ion trap mass spectrometer, which was used for reaction monitoring, the microchip was positioned to a 5-mm distance from the MS entrance cone. Ultra scan and positive ion modes were used and drying gas temperature was set to 150 °C with a flow rate of 2.0 L/min. No nebulizer gas was used. A multitip ion source chip was attached to a rotating platform (Thorlabs CR1-Z7/M, Thorlabs Sweden AB, Gothenburg, Sweden). The rotating platform was controlled by Thorlabs computer software APT motor controller.

Each sample was pipetted onto an unused tip, thus minimizing cross-contamination between samples. After measurements the whole µPESI microchip was cleaned with a Piranha solution (3:1 mixture of ammonium hydroxide (NH_4OH) and hydrogen peroxide (H_2O_2) in an ultrasonic bath, whereafter it was rinsed with deionized water and methanol and let to dry in room temperature. After this cleaning procedure the microchip was ready to be reused.

2.4. Organic synthesis and sampling

The synthesis of tropones from heptafulvenes has been previously described [31]. Briefly, the oxidative cleavage of the semicyclic carbon–carbon double bond of the starting heptafulvene (A, Fig. 3) was studied. The purity of starting heptafulvene was checked with NMR before reaction and no impurities were found. Reaction was carried out in a round-bottomed flask placed in an acetone–ice bath (−15 °C). Heptafulvene A (6.00 mg, 1.0 equiv.) was dissolved in dichloromethane (2.0 mL), producing a 10 mM reaction solution of *m*-chloroperoxybenzoic acid (*m*-CPBA, Aldrich, 77%, 11.0 mg, 2.5 equiv.) to initiate the oxidation reaction. From the magnetically stirred reaction mixture, a 10-µL aliquot was taken and diluted with 1 mL of 0 °C (ice cold) 95% methanol/1% formic acid/4% water by shaking by hands for a few seconds. 1.5 µL of the diluted sample was introduced to the µPESI tip for immediate analysis of remaining starting materials, reaction intermediates and products. The whole sampling procedure took about 10–15 s. The first four samples were taken with a rate of one sample in 1 min and the last one was taken after 10 min of adding the oxidizing agent to a reaction mixture.

2.5. Urine sample pretreatment

A spiked pooled urine sample was prepared to contain nordiazepam, oxazepam, temazepam, α-OH-alprazolam, and α-OH-midazolam with concentration of 200 ng/mL of each compound. Solid-phase extraction of urine sample was done with 10-µL C_{18}-ZipTip^{TM} pipettes (Millipore, Molsheim, France) (Fig. 2d). First, the tips were conditioned three times with 10 µL of acetonitrile and three times with 10 µL of 10% aqueous acetonitrile keeping the tip wet the whole time. After conditioning, the tip was loaded five times with 10 µL of the sample, washed ten times with 10 µL of 10% aqueous acetonitrile, and after the last washing step the tip was let dry. Elution of the sample directly to the microchip was made with 2 µL of 95% methanol/1% formic acid/4% water.

2 mL of authentic positive benzodiazepine urine samples were enzymatically hydrolyzed using 500 µL of 800 mM sodium phosphate buffer (pH 7.0) with 20 µL of β-glucuronidase (*E. coli*, Roche Diagnostics GmbH, Mannheim, Germany). The samples were

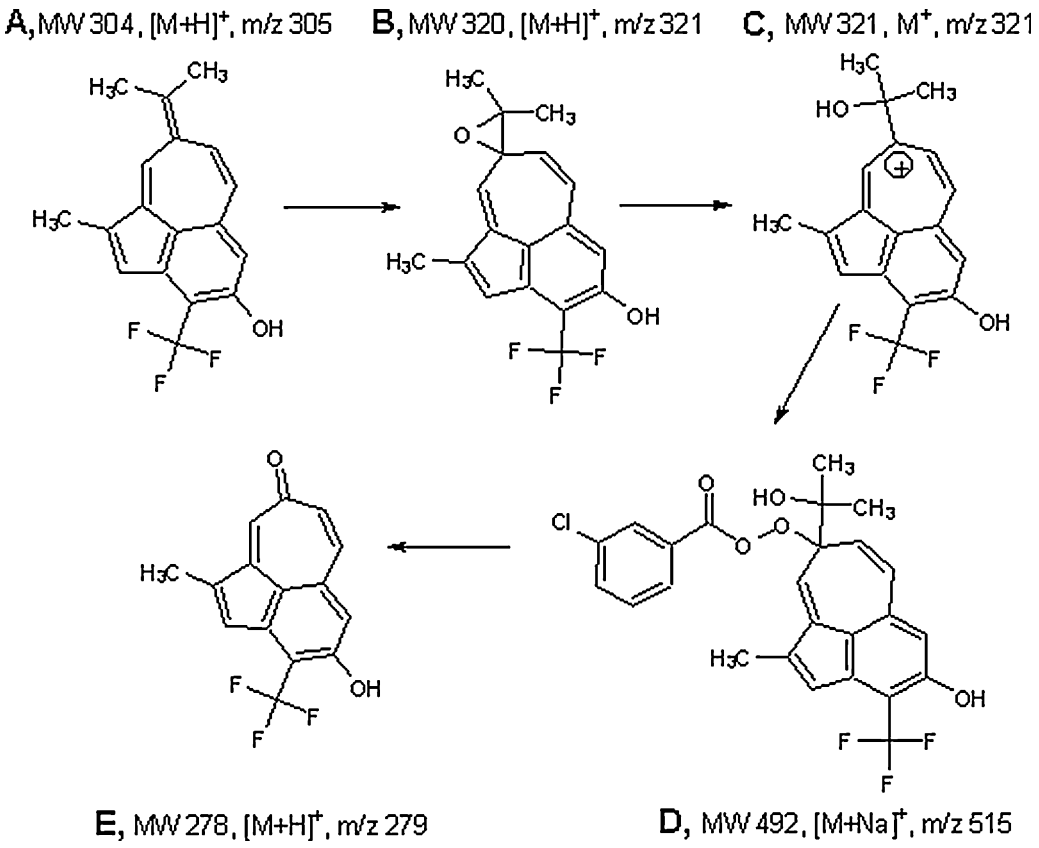

Fig. 3. A synthesis reaction pathway and structures of the intermediates [31] and final reaction products, showing their molecular weights and the *m/z* values of ionized molecules.

vortexed and incubated in 55 °C water bath for 30 min. After that the same ZipTip-solid phase extraction method was used for pretreatment as with spiked urine samples.

3. Results and discussion

3.1. Rapid analysis of reaction products from organic synthesis

The multitip-μPESI with an ion trap MS was applied to monitoring of reaction products from a synthesis of tricyclic tropones from heptafulvenes which has been previously published but the structures of the intermediate products have not been confirmed yet [31]. The synthesis is rather rapid, as the whole synthesis is completed in 10 min. However, despite being carried out as a "one-pot" process, it consists of several chemical reaction steps, thus yielding intermediate products whose structures have not been confirmed so far with any analytical methods. Therefore, the new multitip-μPESI chip was found to be a suitable choice for the analysis as it provides rapid analyses, hence kinetics could also be determined from the analytical results. Due to high concentration of the starting material, and hence of the reaction products, a liquid sample from the reaction vessel was first diluted with methanol by 100-fold prior to the analysis to avoid overloading of the analytical instrument and especially ES ionization. Dilution was made with a chilled solvent to prevent the reaction from proceeding further before the analysis.

The mass spectra obtained from the experiments supported the postulated synthesis route (Fig. 3a) [31]. As an example, a mass spectrum of synthesis products measured at 2.5 min after starting the reaction is shown in Fig. 4a. The starting compound A was observed at *m/z* 305, intermediates at *m/z* 321 and 515 and the final product at *m/z* 279. The ions at *m/z* 337 and 353 are dioxygenated and trioxygenated byproducts of the starting compound A, respectively, and the ion at *m/z* 173 is protonated *m*-CPBA. The ion at *m/z* 321 may represent two compounds, namely the protonated molecule of an oxidation product of A and a tropylium cation. The ion at *m/z* 515 is a sodium adduct of the intermediate D. The ^{37}Cl isotope ion at *m/z* 517 confirmed the presence of one chlorine atom in the molecule. The ion at *m/z* 391 was formed also in the experiment without the starting material A and its origin remained unclear, even with MS/MS data obtained. The ion at *m/z* 286 is a fragment ion from D, produced by the loss of *m*-CPBA and HF, as evidenced in MS/MS measurements (Table 2). The identity of product D has not been previously confirmed with any other method due to its relatively rapid reactivity to form a product E. The structures of the products were confirmed by MS/MS spectra (Table 1). All synthesis products showed an ion $[M+H-HF]^+$ confirming that the products contained at least one fluorine atom (in-CF_3 group) and are derived from the starting material A. The intermediates B/C showed a loss of formaldehyde, producing an ion at *m/z* 291 ($[M+H-CH_2O]^+$) which confirmed that these intermediates contained at least one oxygen close to the isopropyl group. The intermediate D showed a loss of both *m*-chlorobenzoic acid and *m*-CPBA, confirming that the starting material A has reacted with *m*-CPBA. The final product E showed a specific loss of carbon monoxide (CO), producing a six-membered ring and confirming the existence of a ketone group in the molecule. An increase and/or a decrease of the starting material and reaction products as a function of time are shown in Fig. 4b. The analytical cycle was fast enough for the measurement of relative abundances of each reaction product. Interestingly, the multitip-μPESI-MS measurement of the pure starting compound A showed also mono- and dioxygenated products (ions at *m/z* 321 (B) and 337), even without using any oxidant. The starting compound A was known to be highly reactive, thus it could be oxygenated

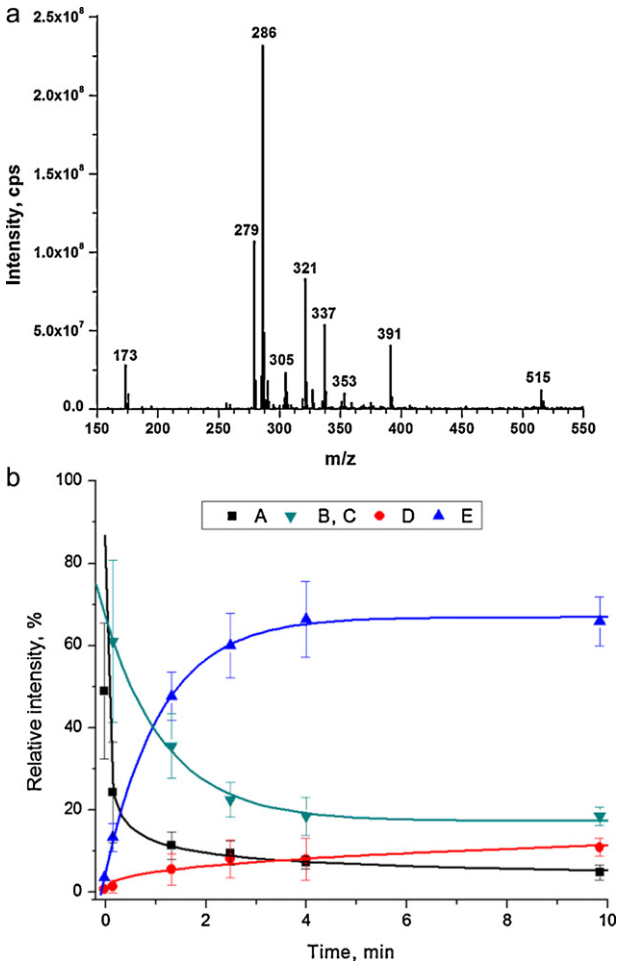

Fig. 4. (a) A mass spectrum of synthesis products measured at 2.5 min after starting the reaction. (b) Relative intensities of the starting compound and the reaction products by a function of time measured by the multitip-μPESI platform with ion trap MS. The RSDs of three replicate experiments are also shown.

Table 1
MS/MS data of the starting compound and reaction products measured by multitip-μPESI platform with ion trap MS.

Compound	Precursor ion, *m/z*	Product ions in MS/MS (*m/z*) and their structures
A	305, $[M+H]^+$	303 $[M+H-H_2]^+$
		289 $[M+H-CH_4]^+$
		285 $[M+H-HF]^+$
		283 $[M+H-H_2-HF]^+$
		269 $[M+H-CH_4-HF]^+$
B/C	321, $[M+H]^+/M^+$	319 $[M+H-H_2]^+$
		301 $[M+H-HF]^+$
		299 $[M+H-H_2-HF]^+$
		291 $[M+H-CH_2O]^+$
		283 $[M+H-HF-H_2O]^+$
		279 $[M+H-H_2-2HF]^+$
D	515, $[M+Na]^+$	495 $[M+Na-HF]^+$
		475 $[M+Na-2HF]^+$
		359 [M+Na-*m*-chlorobenzoic acid]$^+$
		339 [M+Na-*m*-chlorobenzoic acid–HF]$^+$
		286 [M+Na-*m*-CPBA–HF]$^+$
E	279, $[M+H]^+$	259 $[M+H-HF]^+$
		251 $[M+H-CO]^+$
		239 $[M+H-2HF]^+$
		231 $[M+H-CO-HF]^+$
		211 $[M+H-CO-2HF]^+$

already in the solvent by residue oxygen or during ES ionization [32,33]. Another advantage of the new analytical method is the very low amount of the starting compound needed for the analysis (150 pmol ~ 50 ng), therefore the synthesis could be done in a microscale, for example using a microreactor on a chip integrated with an on-chip ESI source. The results obtained in this experiment show that the multitip-μPESI-MS can be used for easy, convenient, and rapid monitoring of organic reactions.

3.2. High-throughput screening of drugs

Screening of drugs from urine samples is commonly performed with immunological methods, but the drawback of those methods is that they can give false positive or negative results because of the lack of the specificity or sensitivity. Gas chromatography–mass spectrometry (GC/MS) [34,35] and liquid chromatography–mass spectrometry (LC/MS) [36,37] are more selective and sensitive methods for screening, but they are still relatively slow for high-throughput screening analysis. Therefore, the feasibility of the multitip-μPESI platform combined with a TOF/MS instrument was evaluated in this study for screening of selected benzodiazepines from urine samples. For sample treatment a rapid ZipTip based method was chosen to take advantage of the fast analytical method. The purpose was also to minimize the total volume of urine used, and it was noticed that 50 μL of urine sample was enough to achieve sensitivity level required for detection of drugs at cut-off concentrations (200 ng/mL) used in immunological screening methods. The analytes were eluted with 2 μL of 95% methanol with 1% of formic acid which was directly deposited on the μPESI sampling spot and subsequently analyzed with MS. Temazepam, oxazepam, nordiazepam, α-OH-midazolam, and α-OH-alprazolam are commonly used target compounds in the screening of benzodiazepines in urine samples, and therefore they were selected for the ZipTip-multitip-μPESI-TOF/MS analysis [38].

The compatibility of the ZipTip-multitip-μPESI-TOF/MS for screening of benzodiazepines was demonstrated with an analysis of spiked urine samples and 12 authentic urine samples, previously detected as positive samples with immunological and GC/MS methods. In the same analysis sequence also standard solutions at cut-off concentration levels (200 ng/mL) and negative control samples (unspiked urine samples) were analyzed. Fig. 5a shows a mass spectrum of a spiked urine sample measured with ZipTip-multitip-μPESI-TOF/MS. The spiked urine sample contained benzodiazepines, namely nordiazepam, oxazepam, temazepam, α-OH-alprazolam, and α-OH-midazolam with a concentration of 200 ng/mL. All benzodiazepines were detected with adequate signal intensity for positive identification when a mass spectrum of a reagent blank was subtracted from the mass spectrum of the sample. Fig. 5b shows a typical mass spectrum measured with the ZipTip-multitip-μPESI-TOF instrument from an authentic urine sample that contained oxazepam (a protonated molecule at *m/z* 287) and α- or/and 4-OH-midazolam (protonated molecule at *m/z* 342). The ions at *m/z* 289 and 344 were concluded to be protonated oxazepam and α- or/and 4-OH-midazolam with a ^{37}Cl isotope, respectively. The sample was previously analyzed with GC/MS and the same compounds were detected, showing the capability of ZipTip-multitip-μPESI-TOF method for high-throughput screening of benzodiazepines from urine samples. The sample treatment with ZipTip extraction was effective as the signals from matrix components were minimal, as shown in the mass spectrum of a blank urine samples (Fig. 5c). The blank urine sample contained some matrix compounds, but the ions detected did not interfere with the analysis of benzodiazepines as they had clearly different accurate masses from those of the analytes. The urine samples were also analyzed directly with multitip-μPESI-TOF/MS without the ZipTip extraction but as assumed, the matrix components interfered with

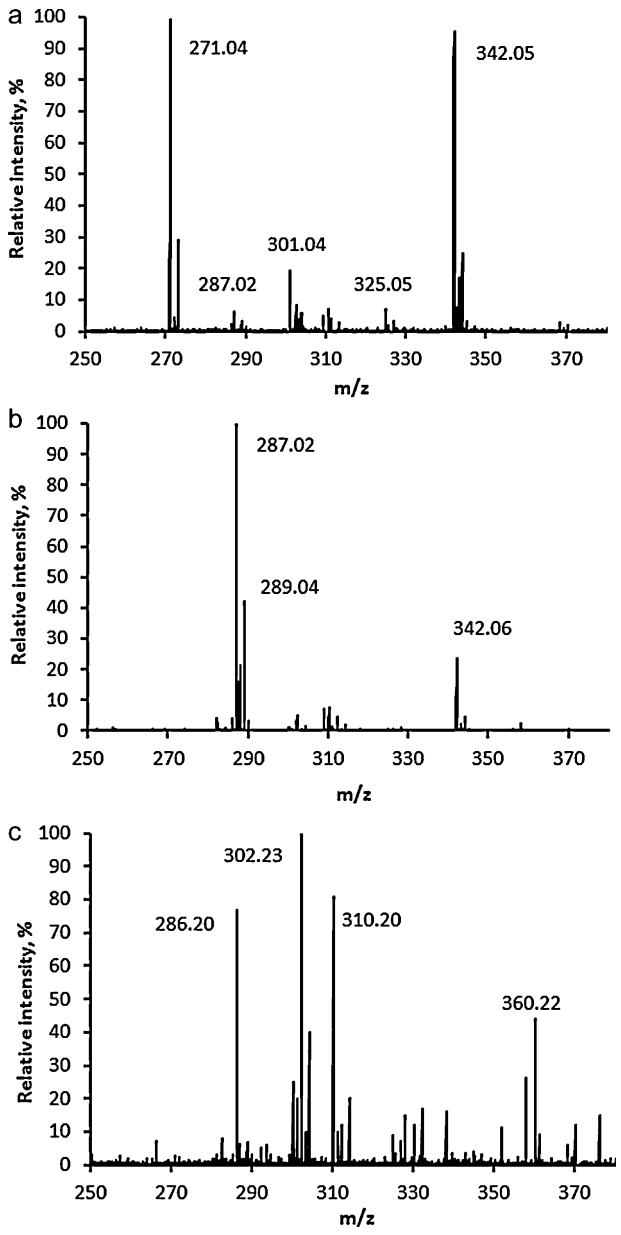

Fig. 5. (a) A mass spectrum of a spiked urine sample containing nordiazepam (*m/z* 271), oxazepam (*m/z* 287), temazepam (*m/z* 301), α-OH-alprazolam (*m/z* 325), and α-OH-midazolam (*m/z* 342) with a concentration of 200 ng/mL, (b) a mass spectrum of an authentic positive urine sample containing oxazepam (*m/z* 287) and α- and/or 4-OH-midazolam (*m/z* 342), and (c) a mass spectrum of a blank urine sample. All samples were analyzed with ZipTip-multitip-μPESI-TOF/MS. Background (a mass spectrum of a reagent blank) was subtracted from the mass spectra A and B.

the ionization and detection of the analytes which were not visible in the mass spectra measured (data not shown).

All authentic urine samples containing benzodiazepines or their conjugates were detected as positive with the ZipTip-multitip-μPESI-TOF/MS method using a detection window of ±20 mDa for identification (Table 2). In some urine samples the concentrations of individual benzodiazepines determined by GC/MS were actually below the cut-off values, therefore it was expected that these compounds would not necessarily be detected by ZipTip-multitip-μPESI-TOF/MS method. No false positives were observed from negative control samples with the method. The results obtained were in a good agreement with those obtained with the GC/MS method, except in the case of oxazepam and one sample (no. 8) with temazepam. Oxazepam was detected in five samples but was

Table 2
Analytical results of benzodiazepines analyzed with quantitative GC/MS and screening ZipTip-multitip-μPESI-TOF/MS methods. Compounds with concentrations below the cut-off value (200 ng/mL) of the ZipTip-multitip-μPESI-TOF/MS are in parenthesis.

Sample	GC/MS, ng/mL	μPESI-TOF/MS, with 20 mDa window
1	α-OH-alprazolam, 2494	α- and/or 4-OH-alprazolam
2	Oxazepam, 396 (desmethyldiazepam, 155) (temazepam, 193)	Desmethyldiazepam
3	α-OH-alprazolam, 462 Oxazepam, 206 (desmethyldiazepam, 32) (temazepam, 26)	α- and/or 4-OH-alprazolam
4	α-OH-midazolam, overload Desmethyldiazepam, 7499 Temazepam, 4601 Oxazepam, 4409	α- and/or 4-OH-midazolam Desmethyldiazepam Temazepam
5	α-OH-midazolam, 1486 (desmethyldiazepam, 164) (oxazepam, 152) (temazepam, 25)	α- and/or 4-OH-midazolam Desmethyldiazepam
6	Oxazepam, 10000 α-OH-midazolam, 164	Oxazepam α- and/or 4-OH-midazolam
7	Temazepam, 67207 Oxazepam, 20292 Desmethyldiazepam, 3150	Temazepam Oxazepam Desmethyldiazepam
8	Desmethyldiazepam, 455 Oxazepam, 1052 Temazepam, 551	Desmethyldiazepam
9	Oxazepam, 397 (desmethyldiazepam, 57) (α-OH-alprazolam, 171) (temazepam, 33)	Desmethyldiazepam
10	Oxazepam, 33810 α-OH-midazolam, 946 (α-OH-alprazolam, 189)	Oxazepam α- and/or 4-OH-midazolam α- and/or 4-OH-alprazolam
11	Temazepam, 4065 Desmethyldiazepam, 220 Oxazepam, 2377	Temazepam Desmethyldiazepam Oxazepam
12	Desmethyldiazepam, 244 Oxazepam, 312 Temazepam, 1801	Desmethyldiazepam Oxazepam Temazepam

not detected in other five samples, due to either ion suppression caused by matrix components or other benzodiazepines, low recovery of oxazepam during the extraction (not likely as it can easily been detected from spiked samples) or decomposition of oxazepam during thaw and freeze cycles as the urine samples could not be analyzed with the ZipTip-multitip-μPESI-TOF/MS method at the same time as with the other methods. However, all samples were detected as positives with the ZipTip-multitip-μPESI-TOF/MS method and in routine analysis all positive screening results would be confirmed with a quantitative analytical method, such as GC/MS or LC–MS/MS techniques.

The whole analytical procedure (SPE and μPESI-TOF/MS measurement) was rapid, as the total analysis time was approximately 5 min per sample. In addition, the consumption of samples and reagents was minimal, as only 50 μL of the urine sample was needed and less than 200 μL of solvents were used in SPE per sample. This new approach could be used in the future as a new alternative for conventional screening methods.

4. Conclusions

Parallelization of the μPESI tips to a rotating multitip platform combined with MS was shown to be a promising method for high-throughput screening in qualitative and semi-quantitative analyses. The speed and easiness of the rotating multitip μPESI platform with ion trap MS proved to be adequate for rapid monitoring of reaction products in an organic synthesis. The amount

of the starting compound needed for the analysis was at a pmol range, which enables utilization of microreactors for the synthesis, still providing high sensitivity in the analysis. The rotating multitip μPESI/MS combined with ZipTip™ solid-phase extraction method proved to be efficient approach for specific screening of benzodiazepines from urine, being rapid, sensitive, and selective. The next step in the development is to automate both the ZipTip™ SPE and μPESI/MS analysis which would reduce the total analysis time from the current five min per sample to approximately one min per sample. This should also further enhance sensitivity and reproducibility of the measurements.

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