CONF-900724--7

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DE90 009423

*Research sponsored by the National Cancer Institute, Interagency Agreement 0485-0485-A1, U.S. Department of Energy contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

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The measurement of nicotine and cotinine in physiological fluids (urine, blood serum, and saliva) is widely used as a means of assessing human exposure to environmental tobacco smoke (ETS). Although numerous analytical methods exist for these measurements, they generally involve extensive sample preparation which increases cost and decreases sample throughput. We report the use of thermal desorption directly into an ion trap mass spectrometer (ITMS) for the rapid determination of nicotine and cotinine in urine. A 1 μL aliquot of urine is injected into a specially designed inlet and flash vaporized directly into an ITMS through an open-split capillary restrictor interface. Isobutane chemical ionization is used to generate $(M+H)^+$ ions of the analytes and collision induced dissociation is used to generate characteristic fragment ions which are used to confirm their identity. Quantification is achieved by integrating the ion current for the characteristic ions and comparing with an external working curve. Detection limits are approximately 50 pg per analyte and the sample turnaround time is approximately 3 minutes without the need for extensive sample preparation.

INTRODUCTION

Because nicotine is a tobacco specific compound and a major constituent of tobacco smoke, its presence (along with its metabolites) in physiological fluids is frequently used as a biological indicator for assessing uptake and exposure to tobacco smoke (1,2). Several analytical methods have been developed for the determination of nicotine and its major metabolite cotinine, in urine, saliva, and blood plasma. These generally involve some type of extraction procedure in order to concentrate and remove the analytes from the matrix, followed by gas chromatographic separation and specific detection with a nitrogen detector (3,4) or a mass spectrometer (5,6). Additionally, liquid chromatography (7,8) and radioimmunoassay (9,10) have also been successfully used. A problem with these methods is that they are labor intensive and time consuming, often requiring at least 30 minutes per determination. As an alternative approach, we have been investigating the use of ion trap mass spectrometry for the rapid determination of nicotine and cotinine in human urine without the need for chromatographic separation and with minimal sample preparation and handling.

Although ion trap mass spectrometers have been in existence for many years, only recently have significant advances in the technology enabled the development of versatile and reliable instrumentation (11,12). State-of-the-art ion trap mass spectrometers have several important features that enable them to be used for the determination of trace compounds

in complex samples. These include very low detection limits, the ability to selectively store or eject ions from the analyzer and the capability of generating full scan CID fragmentation MS/MS spectra on only a few pg of a target analyte. Additionally, with ion trap instruments, the ability to store ions for hundreds of milliseconds enables the use of a variety of chemical ionization reagents which cannot be used with conventional mass spectrometers. This greatly increases the ability to perform selective chemical ionization reactions as a means of improving compound specificity and reducing interferences from matrix ions. Finally, the ITMS instrument exhibits a remarkable tolerance for operation at relatively high pressures which decreases the complexity of sample introduction devices and minimizes adverse effects from sample matrix on the performance of the mass spectrometer.

The method that we describe for the measurement of nicotine and cotinine in human urine simply involves the direct injection of microliter quantities of urine into an ITMS through a specially designed sample introduction system. The determination is performed by means of chemical ionization and tandem mass spectrometry of the target analytes. No extensive sample handling or preparation is required and the time per determination is only 3 minutes. This not only greatly increases the sample throughput but also reduces the labor and cost per analysis significantly.

MATERIALS AND METHODS

All experiments were performed with a Finnigan MAT ITMS ion trap mass spectrometer equipped with a custom designed vacuum chamber which is electro-polished on the inside and pumped with two 330 L/sec turbomolecular pumps. This configuration was chosen in order to minimize contamination of the mass spectrometer by the adsorption of analytes onto the surfaces of the vacuum chamber. Internal infrared heaters maintain the vacuum chamber at 120°C which further reduces background contamination and carry-over from previous samples. The ITMS is also equipped with all of the electronic components necessary for the selective ejection or storage of ions in the analyzer cell as well as for performing collision induced dissociation (CID) tandem mass spectrometry (MS/MS). An IBM AT compatible computer and commercially available software is used to control all of the instrument parameters during data acquisition. Instrument scan functions which enable the specific detection of nicotine and cotinine were written by the authors using the scan function editor program provided with the ITMS.

The sample introduction system consists of a specially designed thermal desorber which was constructed in our laboratory and is depicted in Figure 1. It was designed primarily for the rapid vaporization of samples trapped on sorbent tubes; however, by incorporation of a septum in the cap of the desorber, it may also be used for the direct injection and flash vaporization of liquid samples. During operation, the desorber is continuously purged with a flow of helium which carries volatilized compounds into a capillary restrictor open-split interface. The interface consists of a 1.2 meter length of 180 micron uncoated capillary restrictor which projects directly into the ITMS analyzer cell through a heated sheath. The gas flow rate into the ITMS through the restrictor is approximately 0.5 standard mL/min. The atmospheric pressure end of the restrictor is connected to the output of the thermal desorber through a simple splitter which is adjusted for a split ratio of approximately 80:1. A GC oven was used to maintain the splitter at a constant temperature of 200°C.

Urine for the generation of calibration curves was prepared by spiking urine collected from non-smokers with know quantities of free-base nicotine and cotinine dissolved in methanol. Anhydrous nicotine and cotinine were obtained from Aldrich Chemical Co. and Sigma Chemical Co. respectively. Methanol standards were stored in a freezer and spiked urine samples were used immediately after preparation. No pH adjustments were made to the urine prior to analysis.

The experimental conditions for the determination of nicotine and cotinine included the injection of 1 μL of spiked urine through the septum of the thermal desorber into a 3" long x 0.25" OD glass tube packed with 1 cm of Tenax. The initial temperature of the Tenax trap was approximately 50°C and after a 10 sec delay, the thermal desorber was heated for 10 sec with 350 watts of power to a maximum temperature of approximately 200°C. Helium gas was constantly purged through the desorber at a flow rate of 40 mL/min which was measured with a bubble-type flow meter.

Compounds vaporized from the urine sample were admitted directly into the ITMS instrument through the open-split capillary interface. The uncorrected background pressure inside the ITMS was 1 x 10^{-5} torr with helium flowing through the transfer line. Isobutane was used as a proton transfer chemical ionization reagent and was added through a batch inlet into the ITMS. The partial pressure of the isobutane was approximately 5 x 10^{-5} torr as measured with an ionization-type pressure gauge. For the generation of MS/MS spectra, the isobutane reagent gas was ionized for 5 msec and allowed to react with neutral analytes for 75 msec to form protonated $(M+H)^+$ ions at m/z 163 and m/z 177 for nicotine and cotinine respectively. Using combined RF/DC ion ejection techniques, all ions in the mass spectrometer analyzer cell were ejected except for the protonated nicotine or cotinine. CID fragment ions were generated by irradiating the m/z 163 or m/z 177 ions with 800 mV of resonant RF energy for 10 msec, kinetically exciting the ions and generating fragment ions through collision with neutral molecules in the background. For protonated nicotine, the m/z 163 ion fragments into 4 major ions at m/z 84, 106, 120, and 132, with m/z 106 being the base peak. Protonated cotinine at m/z 177 fragments into 4 major ions at m/z 80, 98, 118, and 146, with m/z 146 being the base peak. For the acquisition of data for a single injection, spectra were repeatedly collected over a 3 minute period of time during which the analytes eluted from the desorber into the ITMS. Beginning approximately 1 minute after the injection of the sample, the desorber was cooled back down to its starting temperature by blowing room temperature air over the device for 2 minutes. Subsequent injections and determinations were made in the same way using the same Tenax trap.

Quantification was performed by integrating the area under the desorption profile for each of the characteristic fragment ions of the analytes. For the data generated in this study, no internal standards were used. External calibration curves from 10 pg-1,000 pg of injected analyte were generated with 3 replicates at each level of 10, 25, 50, 100, 250, 500, and 1,000 pg.

RESULTS AND DISCUSSION

Direct injection thermal desorption profiles of nicotine and cotinine in urine at a concentration of 100 pg/μL each are shown in Figures 2 and 3. Peaks corresponding to the analytes are approximately 15 seconds wide at half height and are readily distinguished

from matrix interferences on the basis of relative ratios of the fragment ion intensities as determined from pure standards. It was found that without the use of the Tenax bed in the thermal desorber, an unacceptably large background was present in the MS/MS spectra which caused a loss of sensitivity. The Tenax not only serves to retain the solids and high boiling constituents which are present in urine, but also provides crude separation of the nicotine and cotinine from the urine matrix during the desorption process. Both nicotine and cotinine are eluted from the Tenax approximately 20 seconds after the trap is heated, while the major interferences begin to elute a few seconds later as a broad peak.

Through the use of selected ion storage for discriminating against background ions and MS/MS for compound identification, it is possible to detect as little as 10-20 pg of either nicotine or cotinine in a 1 μL sample of urine. Reliable identification and quantification is possible at a level of 50 pg. Again it is emphasized that no extraction or chromatography is performed on a sample before or during the analysis.

Working curves for nicotine and cotinine are shown in Figures 4 and 5. Both curves are linear over the entire range from 10-1,000 pg and reproducibility for replicate samples is on the order of 10-15%. Although not shown on these working curves, linear responses have been demonstrated up to 5,000 pg/μL for both compounds. This range of linearity easily encompasses the nicotine and cotinine concentrations that are typically found in the urine of active smokers (approximately 150-3,500 pg/μL) and should also be adequate for the determination of these compounds in the urine of non-smokers exposed to environmental tobacco smoke (approximately 10-100 pg/μL). In order to improve the response and reproducibility for the lower levels found in passive smokers, it should be possible to inject more than 1 μL of sample (5-10 μL) into the thermal desorber or to adsorb larger quantities of urine on Tenax off-line.

CONCLUSIONS AND RECOMMENDATIONS

Direct injection thermal desorption ITMS is a rapid method for the determination of nicotine and cotinine in urine. Using a single thermal desorption device, the sample turnaround time is approximately 3 minutes. Automation of the analysis with a multi-sample thermal desorber could reduce the time per determination to as little as 1 minute by eliminating the time required for cooling the thermal desorber between injections. The instrumental detection limits for reliable quantification are at least 50 pg of free-base nicotine or cotinine injected into the desorber and as little as 10 pg can be identified in a sample. Efforts are currently being devoted to the validation of the method by simultaneous analysis of samples using a standard gas chromatographic procedure.

In order to increase the utility of this method for assessing exposure to nicotine, methods are also being developed for the determination of a wider range of nicotine metabolites including nicotine-n-oxide, cotinine-n-oxide, trans-3'-hydroxycotinine, and glucuronic acid adducts. Further improvements in absolute detection limits are also being investigated by injecting larger aliquots of urine and by preconcentration of urine samples on sorbent traps.

Finally, in order to improve the precision of the method, stable isotope labeled nicotine and cotinine are being investigated for use as internal standards. This should significantly decrease errors due to irreproducible injection and matrix effects.

FIGURES

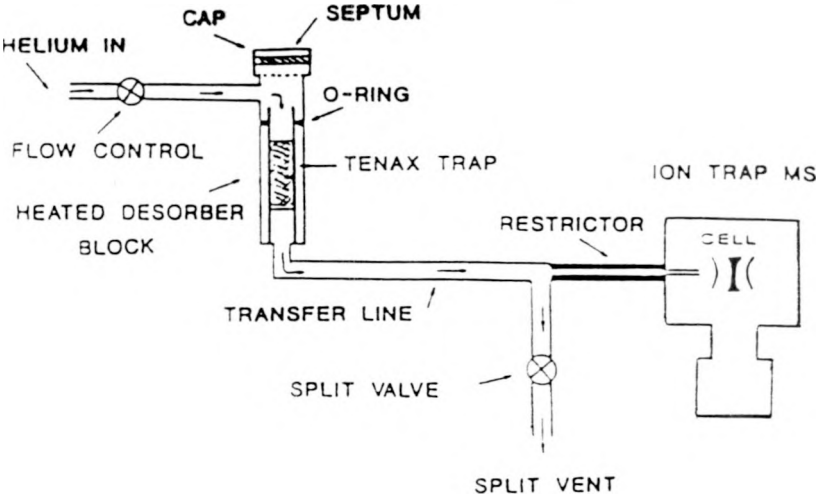

Figure 1: Appparatus for direct injection into the ITMS.

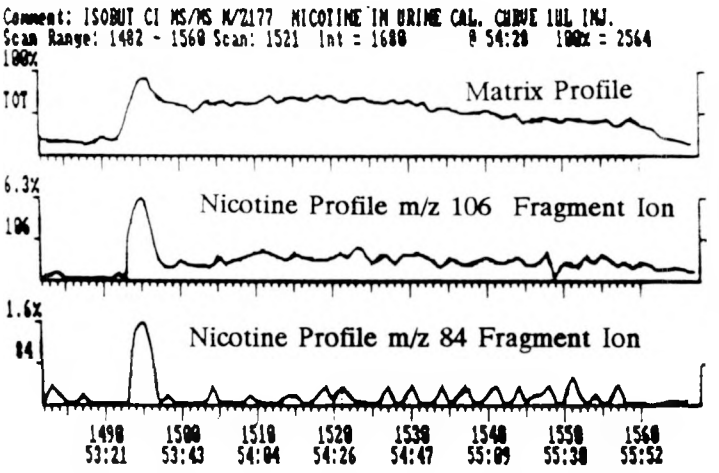

Figure 2: Nicotine desorption profile.

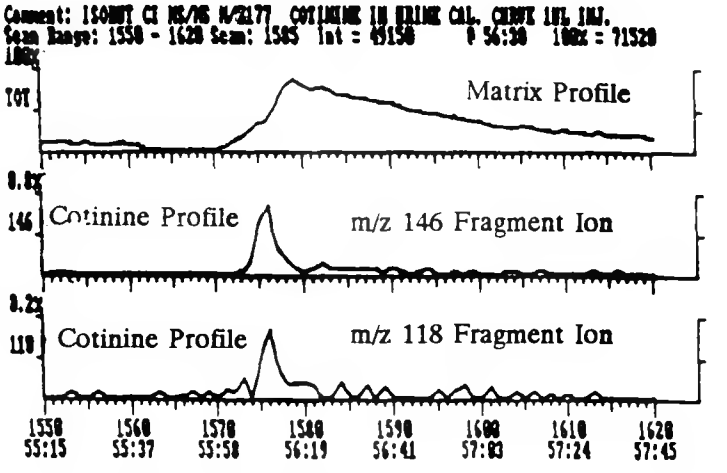

Figure 3: Cotinine desorption profile

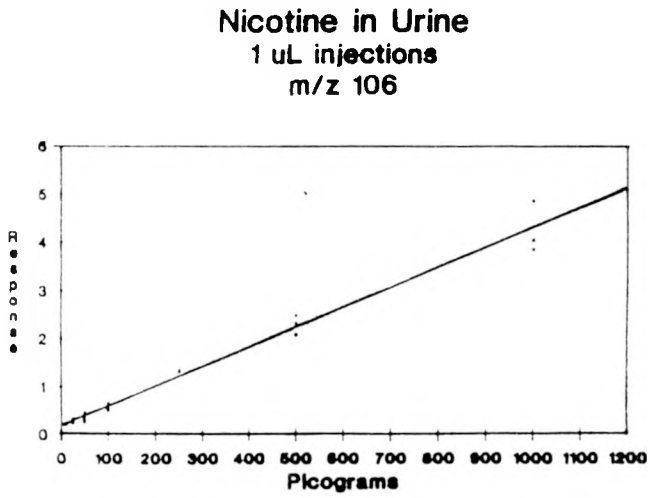

Figure 4: Nicotine working curve.

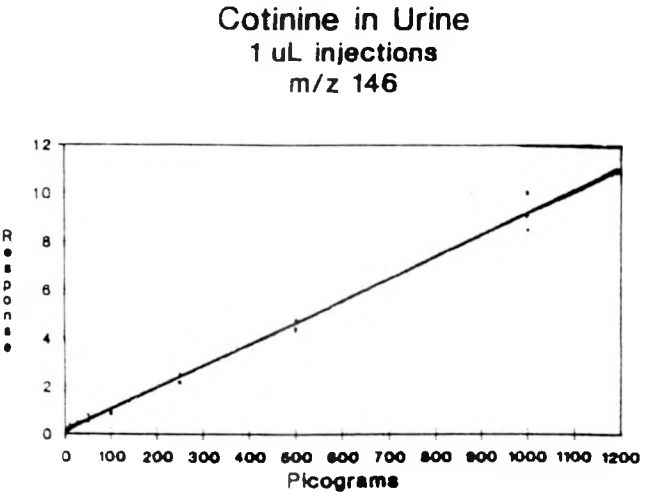

Figure 5: Cotinine working curve.

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