Mushrooms that contain or are suspected to contain the hallucinogenic indole alkaloids, Psilocybin and Psilocin (Figures 1-2) are commonly referred to as “Magic Mushrooms”. These include Psilocybe cubensis, P. mexicana, P. subcubensis, P. azurescens, P. cyanescens, P. semilanceata, P. ovoideocystidoides, and P. pelliculosa. They are naturally occurring and have been used for centuries in the religious ceremonies by shamans in Central and South America. Currently, they have been used extensively for recreational purposes as hallucinogenic substances in various countries in Europe, America, and even in Japan. The hallucinogenic ingredients Psilocybin and Psilocin were isolated by Hofmann et al. in 1958. They have structural similarity to the neurotransmitter serotonin, and their highly hallucinogenic potency is thought to occur from their influence on the serotonergic nervous system. The contents of psilocybin and psilocin in “Magic Mushrooms” have been reported to vary over a wide range from 1-2.5% in a dried mushroom. This wide variation has sometimes resulted in hallucinogenic intoxication by overdosing on “Magic Mushrooms”.

Psilocybin and Psilocin were obtained from Alltech (Deerfield, IL), ethyl morphine (IS) from Cerilliant (Roundrock, TX), and formic acid (99%) from Acros Organics via Fisher Scientific (Pittsburgh, PA). All other solvents were HPLC grade or better and were obtained from Fisher Scientific.

Extraction
Psilocybin/Psilocin and the internal standard (ethyl morphine) were extracted from buffered (pH 6) urine samples (1 mL) utilizing Clean Screen DAU SPE cartridges (UCT, Inc.). After washing the columns (50 µL water and methanol (1:1, v/v) respectively), samples were eluted with methanol and ammonium hydroxide (1:1, v/v). The extract was evaporated to dryness and reconstituted in 200 µL of 0.1% aqueous formic acid. Calibration and quality control standards were prepared in the same manner. A 150 µL aliquot was then injected into the LC-MS/MS system.

Results and Discussion
Linear calibration was established from 10 to 10,000 ng/mL using a six-point calibration curve, and the limits of detection and quantification were 10 to 100 ng/mL, respectively. Recovery values for both psilocybin and psilocin fell at between 95% and 105%, respectively. Intra and inter-day precision was less than 85%. Intra and inter-day precision was less than 5% and 8%, respectively. Standard stock solutions of Psilocybin/Psilocin (1.0 µg/mL) were prepared in acetonitrile. Calibrators were prepared by spiking drug-free urine over the range 10–1000 ng/mL. A stock solution relative to psilocin was determined by spiking the preparation of extraction at 10 and 200 ng/mL. Calibrators and quality control samples were prepared in the same manner as the samples.

Conclusions and Challenges Met
In this methodology, this aim is to develop a SPE procedure in which both psilocybin and psilocin could be efficiently extracted from urine samples. It was concluded that:

1. icus can be extracted from urine at pH 7.4 and isolated with ethyl acetate containing 5% ammonium hydroxide and diethyl ether. A more polar solvent system in tetrahydrofuran containing 1% ammonium hydroxide was used.
2. When psilocybin and psilocin are isolated together, the peak is degraded in the presence of excess base, therefore should be collected under neutral conditions
3. The biological half-life of psilocybin is longer than psilocin, therefore the half-life of psilocin on excision is not as long. The extraction method is simpler and more efficient.
4. The method is rapid and simple, the SPE allows for the extraction of psilocybin but releases Psilocin in the eluent.
5. Extracted samples are stable at 10°C in a LC-MS sample compartment for at least one week.
6. LODs of 20 ng/mL and LOQs of 60 ng/mL of urine were possible by SPE and LC-MS/MS.