

Optimization of Solid-Phase Microextraction Sampling for Analysis of Volatile Compounds Emitted from Oestrous Urine of Mares

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The solid-phase microextraction (SPME) technique was applied and optimized for collection of volatile compounds emitted from oestrous urine of mares *Equus caballus* L. (Perissodactyla, Equidae) for GC-MS analyses. Variables such as type of SPME fibre, collection time of volatiles, and addition of salt were optimized to improve the sampling efficiency in two aspects: extent and selectivity of absorption/adsorption of urine volatiles onto SPME fibres. The data revealed that the number of volatiles and the total amount represented as quantitative peak areas of the compounds trapped on fibres coated either with polydimethylsiloxane-divinylbenzene or with divinylbenzene-carboxen-polydimethylsiloxane were significantly higher compared to those coated with polydimethylsiloxane, polyacrylate, and carbowax-divinylbenzene. The polydimethylsiloxane-divinylbenzene-type of fibre coating was chosen for optimization of sampling time and effect of salt addition. Sampling periods lasted for 15, 30, 60, 120, and 240 min. The optimal collection time of volatiles from urine maintained at about 36 °C was 60 min, as the number of compounds detected with amounts sufficient for quantification did not differ significantly from those trapped during longer collection periods. No significant increase in total amount of volatiles trapped was registered after 120 min of sampling. Addition of 0.3 g NaCl to the 2-ml of samples shortened the collection period from 60 to 15 min during which almost all compounds were trapped. Addition of salt has a significant effect at all sampling periods taking into consideration the total amounts of volatiles trapped. The total intensities increased about 8, 5, 3, 3, and 2 times at collection periods of 15, 30, 60, 120, and 240 min, respectively, when compare with the ones obtained from the urine samples with no salt addition. In oestrous mare's urine, 139 ± 4 (average number \pm standard deviation) volatile compounds suitable for quantitative analyses were detected compared to 45 compounds collected by the gas-tight syringe method.

Key words: Urine Volatiles, Headspace, *Equus caballus*