**Título:** ANALYSIS OF FORENSICALLY RELEVANT DRUGS IN BLOOD AND URINE BY CONVENTIONAL AND ADVANCED LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY METHODS

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**Descriptores:**

> ABUSO DE DROGAS

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**Resumen:** Summary

The present work intended to evaluate the analysis of forensically relevant drugs in blood and urine by conventional and advanced LC-MS/MS methods.

Chapter I gives a short review of the pharmacology of the drugs studied in this thesis (amphetamines, cannabis and hallucinogens) and their effects on human behavior and performance. Hallucinogens are psychoactive substances that powerfully alter perception, mood, and a host of cognitive processes. They are considered physiologically safe and do not produce dependence or addiction. Their origin predates written history, and they were employed by early cultures in a variety of sociocultural and ritual contexts. Nowadays, cannabis is still Europe¿s most commonly consumed illicit drug as nearly a quarter of all Europeans have tried cannabis in their lifetime. Relaxation, well-being and somnolence are one of the effects of cannabis. Moreover, in parts of Europe, use of amphetamines constitutes an important part of the drug problem. They stimulate the CNS, increasing the heart rates and blood pressure and decreasing appetite, among other effects. LC-MS(/MS) is a widely used method in forensic laboratories, particularly in applications where non-volatile, labile or high-molecular-weight compounds are being analyzed.

Chapter II aim to give an overview of the current LC-MS(/MS) applications in forensic laboratories and detailed information in relation to ionization, ion separation, and ion detection, together with tandem mass spectrometry. Suppression or enhancement of analyte ionization by coeluting compounds is a well known phenomenon in LC-MS(/MS) analysis mainly depending on the sample matrix, the sample preparation procedure, the quality of chromatographic separation, mobile phase additives, and ionization type. Therefore, common sample preparation procedures are protein PPT, LLE and SPE. Optimum sample preparation leads to enhanced selectivity and sensitivity. Detailed information about the recent applications of LC-MS(/MS) to the analysis of amphetamines, cannabis and hallucinogens in blood and urine are included at the end of this chapter. However, sample preparation is often regarded as time-consuming, a laborious work. Recent developments in on-line SPE aspects of high-throughput quantitative bioanalysis of drug and metabolite in biological matrices are described in Chapter III. High-throughput analysis is becoming increasingly important in forensic laboratories. One commercial automated on-line SPE system is the Symbiosis system manufactured by Spark Holland. In on-line SPE sorbents of very small particles are packed in a miniature LC column (cartridge) and higher pressures are applied to distribute the SPE solvents. Switching valves direct the flow to the LCcolumn or waste, as appropriate. In contrast of traditional off-line SPE, several times consuming steps are eliminated. The extracted sample is directly injected to the analytical column by a simple valve switch. As a consequence no sample volume is lost during transfer, thus increasing the overall assay sensitivity. An extensive literature survey is given about the application of this instrument to the analysis of drugs in biological matrices.
Chapter IV presents the objectives of the thesis which are an evaluation of the conventional LC-MS/MS technique and the new trend, on-line SPE-LC-MS/MS (Symbiosis), for the analysis of:
a)multiple hallucinogens, chlorpheniramine, ketamine, ritalinic acid and metabolites in urine using off-line SPE,
b)THC and metabolites in blood with LLE as off-line sample preparation procedure,
c)THC-COOH (main metabolite of THC in urine) by on-line SPE, and
d)7 amphetamines and metabolites in blood and urine also by on-line SPE.
Chapter VI presents the development and validation of a LC-MS/MS method for the quantification of hallucinogens and other related compounds in urine. The method comprises an off-line SPE procedure, evaporation to dryness and reconstitution in mobile phase. The total run time was 20 min. External QCs containing LSD where analyzed within each series of analysis. The method was fully validated and applied to authentic urine samples (containing psilocin, ketamine, norketamine and chlorpheniramine).
Chapter VII describes the validation of the method for the analysis of THC and two of its main metabolites in blood. LLE with hexane: ethyl acetate was applied as clean-up procedure followed by centrifugation, complete evaporation, and reconstitution. The run time was 13 minutes. Two external QCs were used within each series of analysis. The method was completely validated in terms of precision, accuracy, specificity, recovery, matrix effects and stability. Finally the method was applied to authentic blood samples from forensic cases. Chapter VIII focuses on the development and validation of a method using the Symbiosis system for the analysis of THC-COOH in urine (500 ¿L). As the THC-COOH is glucoronized in urine, a previous hydrolysis was carried out using KOH 10 M. Then, the diluted urine was acidified in the LC vials for its direct injection. The method was fully validated and applied to authentic samples from cannabis users.
Another application of the on-line-SPE-LC-MS/MS system is presented in Chapter V.IV for the direct quantification of 7 amphetamines and metabolites in blood and urine. The method, completely validated following international guidelines, required a minimum sample handling: the dilution of 100 ¿L blood or 50 ¿L of urine samples in the aqueous mobile phase, spiked with the IS, directly in the LC vials, previous injection. HLB cartridges were used for sample clean-up. The method was applied for the analysis of blood and urine samples

from a MDMA study, and from forensic cases.
The conclusions in Chapter VI show the advantages of LC-MS/MS and the new trend in on-line SPE. The results demonstrated that although conventional LCMS/MS methods are still robust and confident, on-line SPE coupled to LC-MS/MS is highly effective in terms of time safe and high throughput.
Chapter VII is focused in the future perspectives in relation to LC-MS/MS applications. On-line SPE-LC-MS/MS and the UPLC-MS/MS are potent candidates for the analysis of drugs in conventional and alternative matrices (e.g. oral fluid), in such a way that the innovative chromatographic technologies are re-shaping the ways that separations and sample preparation are performed in high-throughput laboratories.