Comparison of Plastic and Glass Collection and Pipetting of Whole Blood for Cocaine Assay by GC-MS

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Trace level detection of drugs is routinely monitored in toxicology laboratory. Whole blood testing is an essential component in evaluation the nature and the concentration value of the ingested drug prior to initiate the therapy. One of the most important steps in lab working protocol regards collection and pipetting technique for whole blood assay. The aim of the study was to compare the effects of whole blood collection and pipetting technique for cocaine and its major metabolite (benzoylecgonine) in trace level assay. The results evidenced that by using both glass and plastic tips, concentration values were lower when using polymeric tube for collection and plastic tips for pipetting compared to glass tubes and plastic tips. In the case of glass containers, the higher concentrations were obtained when using glass pipette. The lower values can be caused by blood adherence to the inner circumferential surface of the pipette tip.

Keywords: toxicology laboratory, polymeric tube, plastic tips, glass tubes, glass pipette, detection of drugs

Trace level detection of drugs is routinely monitored in toxicology laboratory. Whole blood testing is an essential component in evaluation the nature and the concentration value of the ingested drug prior to initiate the therapy. One of the most important steps in lab working protocol regards collection and pipetting technique for whole blood assay. *In vivo*, cocaine is metabolized to benzoylecgonine and m-hydroxybenzoylecgonine [1]. Blood is one of the most important specimens of toxicological interest and various reports explain the impact of specimen containers, pipetting techniques and manipulation procedures in its analysis [2-7]. When following low concentrations of the parent drug in whole blood, a specific manipulation process is needed otherwise measurement uncertainty is compromised [8-13]. Because plastic materials possess the capacity to retain biological specimen on their surface and glass pipettes are more difficult to manipulate, the sampling technique is representative especially when working with biological matrices [14]. The aim of our study was to evaluate the effect of collection and pipetting technique on cocaine concentration in whole blood assay by using both plastic and glass collection containers and tips.

Experimental part

Materials and methods

Methanol, chloroform, ethyl acetate, methylene chloride, isopropanol, all solvents HPLC purity were supplied by Sigma-Aldrich. Cocaine free base standard was obtained from LGC Standards. Potassium carbonate was purchased from Sigma – Aldrich and benzoylecgonine deuterated from Cerriliant. Plastic tube collection, 4 mL were obtained from MediPlus and glass tube containers were supplied by Eurolab Sp. Zoo. Eppendorf high quality plastic tips were used for the extraction procedure.

Sample collection

Whole blood samples were collected in two different types of sterile single use containers of 4 mL. First type was made of medical polymer materials and products coated with heparin lithium and used together with blood collection needle. Second type of glass material vacuum tube with spray-coated lithium heparin, was used for whole blood collection in clinic and tested in lab.

Pre-analytical phase

Following homogenization, samples were submitted to liquid liquid extraction technique. In order to achieve the highest recovery yield, two mixtures of extraction solvents were tested. A mixture of methylene chloride and ethyl acetate (4:1, v/v) and chloroform: isopropanol (4:1, v/v) were evaluated prior to perform with samples extraction. 50 μL benzoylecgonine (internal standard) and 2 mL potassium carbonate 0.36 M were added to 1 ml whole blood. Samples were vortex mixed and extracted by sonication for 20 min. The extraction was repeated three times with 5 ml extraction solvent. The organic phase was separated by centrifugation, 5 min at 3000 rpm, and evaporated under nitrogen stream at 40°C. The resulted residue was reconstitued in 100μL methanol and submitted to GC-MS analysis.

Separation

The chromatography was performed on 450 GC Varian, using helium as carrier gas at a constant flow rate of 1.2 mL/min, and a linear velocity of 49.5 cm/s. The column used for separation was BR-5ms (30m x 0.25mmID, 0.25μm film), (Brucker Daltonics). Column temperature program was set as following: 50°C, 1 min, raised to 200 °C with 10°C/min, and raised again at 300°C held for 4 min. The temperature of the injection port was set at 250°C.

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Sample no.	Cocaine concentration, µg/ml			
	Glass tube – glass pipetting	Glass tube – plastic tips	Polymer tube-glass pipetting	Polymer tube-plastic tips
1	0.014	0.010	0.007	0.005
2	0.119	0.082	0.067	0.059
3	0.373	0.350	0.299	0.273
4	0.624	0.592	0.530	0.455
5	0.890	0.880	0.768	0.666
6	1.711	1.694	1.665	1.000
7	3.732	3.614	3.560	3.353
8	5.435	5.296	5.159	4.904
9	12.362	11.821	11.650	11.169
10	28.909	28.282	27.520	27.463

Table I
COCAINE DETERMINATION IN WHOLE BLOOD SAMPLES

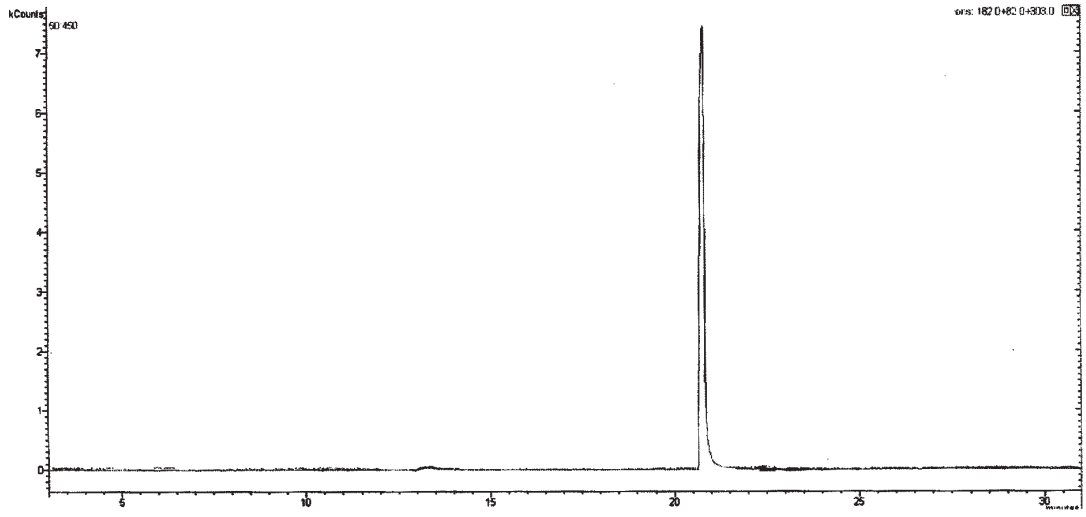

Fig.1. Cocaine separation in whole blood samples under SIS mode

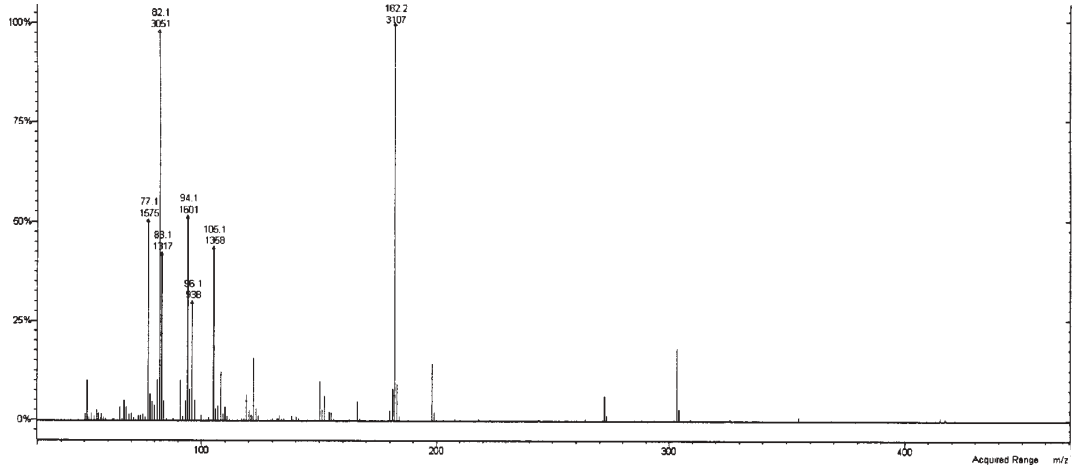

Fig. 2. MS fragmentation pattern of cocaine in selective ion trap mass detector

Samples were injected at a volume of 1 µL using split ratio for 5min, followed by splitless mode 10min. Analysis was achieved in a total run time equal to 30 min.

Identification

Mass spectrometry was performed on 240 MS Ion Trap (Varian). The ionization parameters were set as follows: ion trap temperature at 170°C, the transfer line temperature at 230°C. Ionization was performed in EI mode at 70eV. Nominal mass was used for recording full spectra and single ion storage technique was applied for the identification of cocaine from endogenous compounds of the matrices. Cocaine confirmation was based on fragmentation pattern in ion trap detector and the collected spectrum was correlated with the spectral database (NIST, Wiley, and PMW).

Results and discussions

It is well established that sampling must and can only be optimized prior to the pre-analytical and analytical phase. Both, collection and pipetting techniques represent an extremely important step in order to extract a representative amount of drug from biological matrices. Cocaine separation is presented in figure 1 and MS fragmentation pattern is presented in figure 2. The results showed in Table I evidenced that by using both glass and plastic tips, concentration values were lower when using polymeric tube for collection and plastic tips for pipetting compared to glass tubes and plastic tips. In the case of glass containers, the higher concentrations were obtained when using glass pipette. The lower values can be caused by blood adherence to the inner circumferential surface of the pipette tip.

Conclusions

It was evidenced the effect of collection and pipetting technique on cocaine extraction from whole blood samples. By employing liquid liquid extraction followed by gas chromatography tandem mass spectrometry analysis, it was showed that plastic tubes respectively plastic tips can conduct to a lower concentration of the drug in the case of whole blood manipulation procedures.

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