

# Study For The Development Of Isolation And Clean Up Process By Using Solid Phase Extraction (SPE) For Fluoroquinolones And Cephalosporins Antibiotic Drugs From Forensic Samples

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## Abstract

A new and sensitive sample clean up method is been developed for the extraction of the antibiotic drugs of cephalosporin and fluoroquinolone group from forensic toxicological samples (Blood, Urine and tissues). The method is simple and easy to do in normal routine analysis in the laboratory. . The suggested solid phase extraction (SPE) method is simple and time efficient for easy and fast cleaning of biological samples for quality sample preparation. The C-18 cartridges were used in the process and the average recovery of these cephalosporin and fluoroquinolone antibiotic drugs after sample clean up was >90% for in urine and > 80% in blood and tissue samples for forensic analysis.

## INTRODUCTION

The principle aim of toxicological methods of 'Isolation and Clean Up' is to provide an ideal concentrate to which the chromatographic and other instrumental methods of identification as well as chemical reactions can be applied. Before applying methods of detection and determination in forensic samples, it is necessary to apply appropriate sample clean up procedure which should be simple, economic and result oriented during the examination of biological samples. The various biological samples including mainly Blood, urine, stomach wash, spleen, liver and kidney tissues contains coagulated proteins and large amount of fats which interferes with the chemical activity of target drug present in the matrix, moreover this interference induces improper or no identification of the drugs in the matrix during qualitative and quantitative analysis of any drug in forensic samples. Various authors have used the solid phase extraction procedures [1,2,3,4,5,6] for the extraction of drugs from different types of samples. Under the following study, a sample clean up method is being proposed for the separation of fluoroquinolones and cephalosporin antibiotics in forensic samples. The suggested solid phase extraction (SPE) method is simple and time efficient for easy and fast cleaning of biological samples for quality sample preparation

## MATERIAL AND INSTRUMENTATION

The different biological samples including blood, urine and tissues (Stomach, spleen, kidney and liver) were spiked with known quantity of standard antibiotic drug samples and placed in incubator for overnight to let the interaction of antibiotic sample and the biological matrix. The C18 SPE cartridge, a Solid Phase Extractor (SPE) instrument (Manual operation), acetonitrile, methanol, 2M-perchloric acid, Whatman filter paper No.42 HPLC grade water, a centrifuge and a Nitrogen evaporator is used for clean up and sample preparation process.

## PROCEDURE OF SAMPLE CLEAN UP AND PREPARATION

### FOR CEPHALOSPORIN ANTIBIOTIC DRUGS

A C<sub>18</sub> SPE cartridge was washed with 15ml of methanol followed by 5ml of waters. The extract was passed through the column, packed with C<sub>18</sub> sorbent at a flow rate of 1.5ml/min. The column was washed with 20ml of water. The cephalosporin group antibiotics were eluted with 4ml of acetonitrile: water (3:1 v/v). The elute was evaporated to dryness at 45-50 ° C under a stream of nitrogen. The dried residue is reconstituted by addition of 1000µL water and filtered through a 0.45µm PVDF filter. Now the filtrate could be used for qualitative and quantitative analysis. The

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recovery was calculated by using  $\lambda$ - max absorbance calculations and comparisons.

## FOR FLUOROQUINOLONES ANTIBIOTIC DRUGS

Pre conditioned the SPE cartridge with 5 column volumes (30ml) of deionised water. The pH 4.7 adjusted solutions were loaded into preconditioned cartridges. Load solution has been passed at flow rate of 1ml/min. After load solution has been passed, carefully leave a small amount of liquid above the cartridge bed at all times until final elution. Cartridge was washed with 2ml methanol, followed by 2.5ml methanol-water (1.5:1 v/v) and then 2ml of water is also added. These fractions were discarded. The residues were eluted from the cartridges with 3ml 2M perchloric acid in 2 portions (1ml and 2 ml) into a 15ml plastic centrifuge tube. The filtrate is dried in nitrogen drier and reconstituted for qualitative and quantitative detection of fluoroquinolones antibiotic from biological sample. The recovery was calculated by using  $\lambda$ - max absorbance calculations and comparisons.

## RESULTS AND OBSERVATIONS

The proposed sample clean up procedures was found effective, simple and time saving in the processing of forensic samples. The following table no. 1 and table no. 2 contains the average recovery data of cephalosporin and fluoroquinolones antibiotics from forensic toxicological samples respectively. The data is also presented in chart: 1 and chart: 2.

**Figure 1**

Table 1

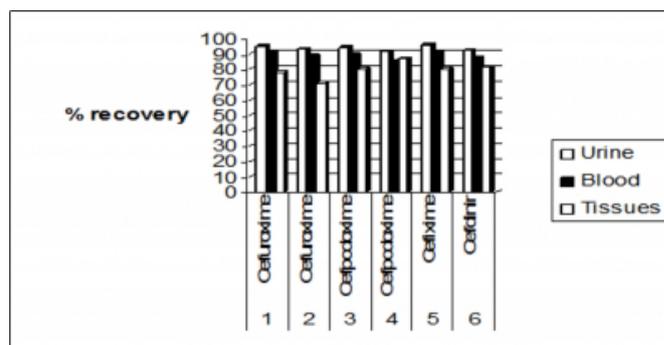
S.No.	Drug Spiked (10 mg/ml) (Cephalosporins)	Recovery in biological samples*		
		Urine	Blood	Tissues
1.	Cefuroxime	95.0	90.8	78.0
2.	Cefuroxime axetil	93.0	88.7	70.8
3.	Cefpodoxime	94.05	89.0	80.2
4.	Cefpodoxime proxetil	91.2	85.0	86.9
5.	Cefixime	96.0	91.0	80.0
6.	Cefdinir	92.0	88.0	81.0

\* Average of five separate determinations

\* Average of five separate determinations

**Figure 2**

Chart 1: Block chart showing the average recovery of the cephalosporin antibiotics from Urine, blood and tissues



**Figure 3**

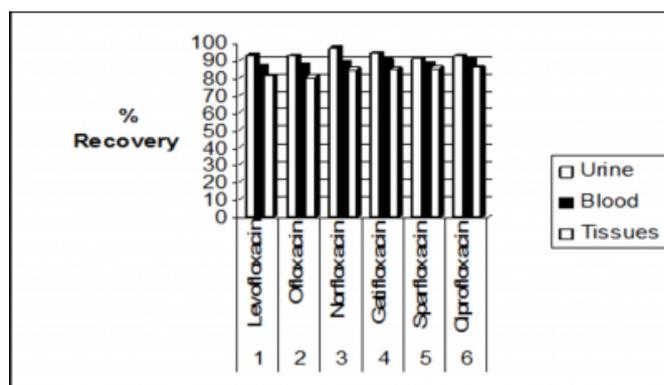
Table 2

S.No.	Drug Spiked (10 mg/ml) (Fluoroquinolones)	Recovery in biological samples*		
		Urine	Blood	Tissues
1.	Levofloxacin	93.0	86.8	81.0
2.	Ofloxacin	92.8	87.4	80.2
3.	Norfloxacin	97.2	89.2	84.5
4.	Gatifloxacin	94.2	89.8	84.8
5.	Sparfloxacin	91.0	88.2	85.4
6.	Ciprofloxacin	92.6	90.4	86.0

\* Average of five separate determinations

**Figure 4**

Chart 2: Block chart showing the average recovery of the fluoroquinolone antibiotics from Urine, blood and tissues



## CONCLUSION AND DISCUSSION

The current study for determining the efficacy of the derived method for the extraction of cephalosporin's and fluoroquinolones has highlighted some positive results in perspective of the recovery of cephalosporin and fluoroquinolone antibiotics from the forensic toxicological

samples. The average recovery of cephalosporin and fluoroquinolone antibiotic from the urine sample was > 90%, from blood and body tissues it was estimated >80-90% which suggests that the proposed method could be utilized as a simple and rugged SPE extraction procedure for these antibiotic classes.

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