A Validated and Applicable Direct Injection LC/MS/MS Method of Fourteen Drugs of Abuse in Urine Samples to Avoid the False Positive/Negative Results of Immunoassay Techniques in Forensic Cases

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1. ABSTRACT

Many false positive and false negative results have been detected in immunoassay analyses of drugs of abuse in urine samples. A method of direct injection of diluted urine into LC/MS/MS was developed and validated for detection and quantitation of Amphetamine, Methamphetamine, MDMA, MDA, Benzylecgonine, Ecgonine, Norpseudoephedrine, Ephedrine, Tapentadol, Tramadol, O-desmethyltramadol, Tapentadol, Pregabalin, Gabapentin and Methadone to avoid the false positive and false negative results in urine samples. Linearity of Amphetamine, Methamphetamine MDMA, MDA, Benzylecgonine, Ecgonine, Norpseudoephedrine and Ephedrine was (60-2400 ng/mL), for Tapentadol, Tramadol, O-desmethyltramadol, and Methadone was (50-1600 ng/mL), and for Pregabalin and Gabapentin was (100-4000 ng/mL) and R² > 0.992 for all analytes. A 440 urine samples have been analyzed using both immunoassay technique and LC/MS/MS by direct injection method giving a good comparison to illustrate how this method was specific, accurate, precise, and applicable for forensic urine samples.

2. Introduction and Conditions

1. LC/MS/MS Liquid Chromatography

The analysis was performed using a Shimadzu 8050 LC-MS/MS triple quadruple mass spectrometer (Kyoto, Japan) coupled with a Shimadzu UPLC Nexera X2 system (Kyoto, Japan). The mobile phase used were used was 0.01 M of ammonium acetate and 0.1 % formic acid in water (A) and 0.01 M of ammonium acetate and 0.1 % formic acid in methanol with gradient mode.

2. Immunoassay

Two immunnoassay instruments were used; (Abbott Archetchit-4000c system, Waver, Belgium), and (V-Twin, Siemens). The kits were used are Pregabalin, Amphetamines, Cocaine (Benzylecgonine), Methadone and Tramadol at cut-off levels; 500, 300, 200, 200, 200 ng/mL respectively according to SAMSHA (5).

3. Sample Preparation

The very easy sample preparation were used, after the centrifugation and filtration of 0.5 µL of urine sample by 0.2 µL PPT filter, 50 µL of urine were diluted by 425 µL of deionized water and 25 µL of IS then 1 µL was injected into LC/MS/MS.

4. Method Validation

The described method was validated in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), specificity, stability, precision and accuracy according to international guidelines on the bioanalytical method validation.

4.1. Linearity and quality control samples.

Only deionized water was used to dilute the standard working solution. For calibration curve, 50 µL from each level of standard working solutions were added to 425 mL of deionized water and 25 µL of IS was added giving 10 fold diluted of the concentration levels of 50, 100, 200, 400, 800, 1200 and 1600 ng/mL for Tapentadol, Tramadol, O-desmethyltramadol, and Methadone, and 60, 150, 300, 600, 1200, 1800 and 2400 ng/mL for Amphetamine, Methamphetamine, MDMA, MDA, Benzylecgonine, Norpseudoephedrine and Ephedrine and 100, 200, 500, 1000, 2000, 3000 and 4000 ng/mL for Pregabalin and Gabapentin at concentration, then 1 µL was injected into LC/MS/MS (Fig. 2). Quality control of analyses were prepared at 100, 200, 300 ng/mL.

Fig. (1): Drugs Under Investigation and the LC/MS/MS Equipment

Fig. (2): Calibration Curves for the Investigated Fourteen Drug Compounds

4.2. Selectivity (Specificity)

Selectivity was studied by analyzing 10 different blank urine samples. No any interferences were observed at the retention time of the analyses and internal standards, (Fig. 3).

While the false negative results, were shown in the low concentration samples due to the high cut-off limits of the immunoassay techniques. In forensic application, these issues should be avoided.

A 440 urine sample was analyzed by the two-immunoassay techniques. A many false positive and false negative results were observed as in (Table 1). The results were confirmed by LC/MS/MS and GC/MS. About (14-20 %) of Amphetamines tests were given false negative results by immunoassay technique, while (6-10 %) were given false positives, about of (17-20%) of Pregabalin tests were given false negative, while (5-6%) were given false positive and about (15-30%) of Tramadol, Benzylecgonine and Methadone were given false negative results, while no false positive were detected of these analyses.

Table (1): RMN Transitions, Retention Time and Compound Tuning Parameters

Table (2): False positive and false negative results of 440 urine samples

III. Results and Discussion

LC/MS/MS technique is the golden solution to avoid these failures of immunoassay technique. The false positive and false negative results were completely avoided in direct injection LC/MSMS method as in (Table 2). The method more specific, more accurate and more reliable than the immunoassay technique.

IV. Conclusion

High numbers of false positive and false negative results came during the analysis of 440 urine samples by immunoassay techniques in forensic applications. These false positive because the high cross reactivity -low selectivity- with the compound structurally like amphetamine and Pregabalin and also because of putrefaction in case of post-mortem cases. False negative results, mainly due to the high cut-off values of the immunoassay techniques. A simple, sensitive and specific, LC/MS/MS direct injection method for determination of Amphetamine, Methamphetamine, MDMA, MDA, Benzylecgonine, Norpseudoephedrine, Ephedrine, Tapentadol, Methadone, Tramadol, O-desmethyltramadol, Pregabalin and Gabapentin in diluted urine samples in forensic application, was developed and validated according to the international guidelines. Use of LC/MS/MS as an alternative screen test, significantly decreased the numbers of false negative and false positive results in forensic toxicology analysis.

V. References

5. Federal Register (Vol. 69, No. 71, Tuesday, April 13, 2004 Mandatory Guidelines and Proposed Revisions to Mandatory Guidelines for Federal Workplace Drug Testing Program; Notices Section 2.4 Laboratory Analysis Procedures (1) (1) confirmation.