LIQUID CHROMATOGRAPHY – TANDEM MASS SPECTROMETRY FOR THE DETERMINATION OF NEW SYNTHETIC OPIOIDS

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In many clinical or toxicology laboratories gas chromatography/mass spectrometry using electron impact ionization (GC/EI-MS) or electron capture ionization, and finally, liquid chromatography with ultraviolet detection (LC-UV) or with tandem mass spectrometry (LC-MS/MS) have been used for comprehensive illicit drugs confirming [1]. Unfortunately, GC/EI-MS is not capable of directly analyzing illicit drugs that are non-volatile, polar or thermally labile. Besides, lengthy sample preparations, which include hydrolysis and derivatization, are required prior to GC/EI-MS analysis [2]. The limitations of LC-UV are well established [2], which include the limited specificity and variability of UV spectra. Also, many illicit drugs have little to no UV absorbance, restricting the menu of detectable drugs. LC-MS/MS method has higher sensitivity and specificity than LC-UV and GC/EI-MS methods [1, 2].

Recently, some clinical or toxicology laboratories have adopted liquid chromatography-mass spectrometry (LC-MS) as a complementary method to LC-MS/MS [2]. However, unlike LC-MS, LC-MS/MS using electrospray ionization (ESI+) is capable of detecting non-volatile, polar and thermally labile illicit drugs and provides a means of detecting a broad range of drugs without the need for lengthy sample preparations. Also, in this study, LC-MS/MS is the analytical method of choice because it's commonly used in forensic toxicology laboratories [1-3].

New synthetic opioids, especially fentanyl analogues are popular in recent years among drug addicts and have been related to many overdoses and deaths worldwide [4, 5]. Therefore, the main aim of my study was to develop a new sensitive and specific method based on LC-MS/MS using solid-phase extraction (SPE) on ChemElute® columns for the determination of new synthetic opioids (fentanyl, norfentanyl, carfentanyl, norcarfentanyl, sufentanyl, norsufentanyl, 3-methylfentanyl, acrylfentanyl, furanylfentanyl, AH-7921, U-47700) in whole blood and urine samples. A diatomaceous earth sorbent was used for SPE of biological samples. The use of extraction solvents for the elution of the adsorbed new synthetic opioids and finally sample pretreatment at different pH values from pH 4.5 to pH 12.0 were also optimized. For the quantitative bioanalytical methods there is a general agreement that at least the following validation parameters should be evaluated [5, 6]: the limit of detection (LOD), quantification (LOQ), selectivity, calibration model (linearity), accuracy, precision (RSD), recovery, robustness and stability. The reliability of this method was certified using an exhaustive validation study.

In this study, liquid chromatography with an efficient separation column (particles size of 2.7 microns) has become the leading separation technique in chromatography due to its flexibility, accuracy, and efficiency. Although liquid chromatography achieved physical separation of new synthetic opioids in a mixture, the MS spectrum offered more information about their structural identity. The addition of tandem MS technology has improved the specificity and accuracy of the detection method, especially for AH-7921 and U-47700 substances. The triple-quadrupole mass spectrometry capability of the selected system has facilitated the simultaneous identification and quantification of all analytes. In the experimental, especially chromatographic conditions and MS/MS parameters were also optimized.

The presented method has several advantages when compared with other previously published data [5-7]. The LOD and LOQ for new synthetic opioids in whole blood and urine samples for the developed method were in the range of 0.01 – 0.10 ng mL^{-1} and 0.03 – 0.25 ng mL^{-1}, respectively. The linear relationships with the coefficient of determination (r2) were in the range from 0.9991 to 0.9997. Furthermore, no interferences were observed from the tested substances at the retention times of new synthetic opioids. The results of intraday and interday accuracy tests for three different concentrations of analyte were in the range of 80.6 – 109.7 %, while all RSDs (precision) for replicate determinations were in the range of 3.28 - 8.64 %. Moreover, it was determined that extraction efficiency ranged from 84.6 (±5.2) % to 96.8 (±3.0) %. The absolute recoveries for most of the new synthetic opioids ranged from 81.8 to 90.4 %, except for AH-7921 (72.4 %) and U-47700 (68.9 %). Moreover, this method has several advantages: elimination of interferences, a multi-residue analysis, and very fast chromatographic separation of eleven analytes, the total run time was 9.5 min.

Obtained results showed, that the developed LC-MS/MS and solid-phase extraction method is accurate, sensitive, selective and specific enough to detect analytes after a long time of a single injection or oral administration of some illicit drugs. Finally, it was demonstrated, that this method is applicable for the determination of trace concentrations of new synthetic opioids in real blood and urine samples. The developed method can be applied in routine toxicological analysis during the investigations of both clinical and forensic cases.

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