Fast LC-MS/MS screening method for the evaluation of drugs, illicit drugs, and other compounds in biological matrices

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INTRODUCTION

The number of cases of severe and fatal poisoning associated with the New Psycoactive Substances (NPSs) consumption has recently increased. Ones of the major limitation in the context of NPSs is related to the fact that appropriate confirmation methods have not yet been developed and validated. Consequently, the negativity of the test could result even if the subject has taken illicit substances. In this work a rapid screening procedure in liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) for the qualitative evaluation of 739 compounds in biological samples (blood, post–mortem blood, and urine) has been described.

MATERIALS and METHODS

STANDARD AND SOLVENTS EUREKA srl LAB DIVISION (code SC9000)	• Mobile phases M1: H_2O + 2 mM ammonium formiate + 0,2% formic acid; M2: Acetonitrile + 2 mM ammonium formiate + 0,2%formic acid • Reagents for proteine precipitation and enzymatic digestion (with glucorinades) • Restek Allure PFPP (5 µm 60Å – 50 x 2.1mm) column
SAMPLE COLLECTION AND STORAGE	• The biological samples, taken through sterile containers (urine), and count tubes (blood and post-mortem blood), followed the current llegal supervision procedures and were stored at -20°C until the analysis
SAMPLE PREPARATION	• Protein precipitation of blood and post-mortem blood (100 µL) → centrifuged → supernatant dilution with aqueous solution (0,1% formic acid - 1:1, v:v) → injection of 30 µL of the diluted sample • Enzymatic reaction of urine (500 µL + 100 µL of glucorinades solution ≥ 100000 U/mL) → incubation for 3 hours at 60°C → dilution with aqueous solution containing the deuterated IS (1:6, v:v) → vortex and injection of 30 µL
LC-MS/MS INSTRUMENTATIONS AND PARAMETERS	• ABSciex API 4500 Qtrap plus Shimazdu Nexera X2 LC system composed by SIL-30AC autosampler, LC-30AD pump, and CTO-20AC column oven • Initial conditions 90% (M1) and 10% (M2), T 40°C (± 1°C) • Ion source mass spectrometer Turbo Spray with curtain gas at 30, ion spray voltage 5400 V, T 550°C, ion source gas 1 at 55 and ion source gas 2 at 60

RESULTS and REAL SAMPLES ANALYSIS

The samples (100 blood and urine samples + 50 post-mortem blood samples) were first analysed with the immunoenzymatically screening methods already in use and accredited (ILabTaurus); all the samples were also analysed with confirmation test to check the obtained screening results. The screening procedure then foresees to compare the MS/MS spectrum of the sample (raw MS/MS) with the spectrum stored in the database (MS/MS database).

Following a matching process, the system provides the chemical structure, information and the best-fit score in order to obtain the correct identification of the compounds.

In **Figure 1** has been shown the chromatogram related to real sample positive to carbamazepine and carbamazepine epoxide.

This procedure was able to meet the needs of a screening method using an LC-MS/MS instrument, allowing chemicals to be identified in an extremely short time (18 minutes per analysis) for a large quantity of molecules (739).

The comparison gives 100% correct identification of the positive and the type of substance.

The method can be also applied for the analysis of cadaveric blood and other unconventional and alternative matrices (e.g. salive, vitreous humor, keratin matrix, etc.).

CONCLUSION

The herein reported method has allowed the indentification of an extremely high number of xenobiotics in a single chromatographic run, by exploiting the potential for signal acquisition given by the hyphenated instrumental configuration.

The method was also applicable without particular problems, as it has been shown to have 100% correct identification and qualification of the substance (diagnostic efficiency), following the comparison of the results obtained with the immunoassay tests and the confirmation analyzes by LC–MS/MS.

During the sample preparation phase, the volume of the sample and the solvents used, as well as the handling of the sample were reduced to a minimum, fully adhering to the principles of Green Analytical Chemistry (GAC).

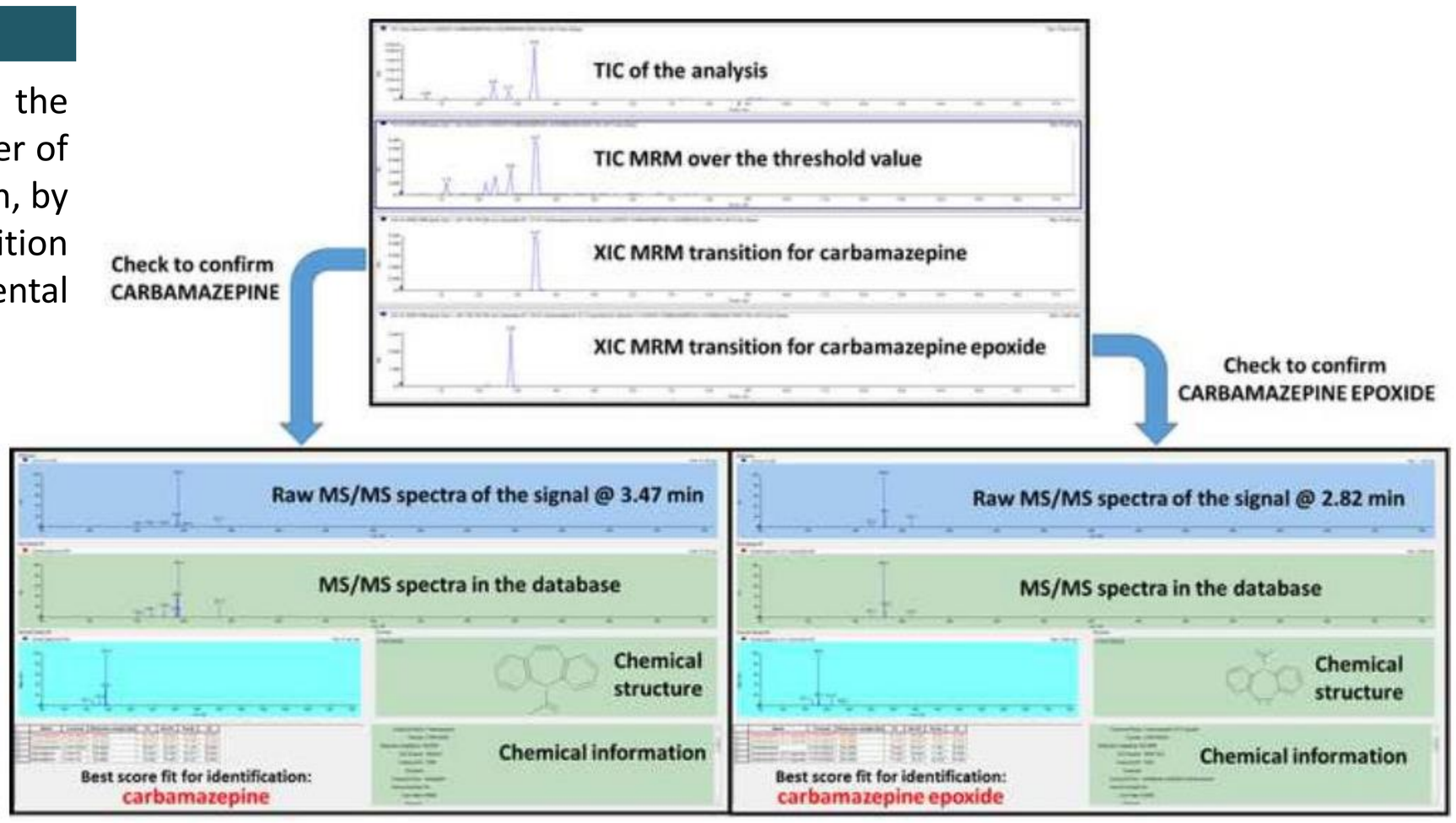

Figure 1 Analysis on a real sample positive to carbamazepine and carbamazepine epoxide.

CONTACT INFORMATION

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Le indagini forensi ed il contributo della spettrometria di massa

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