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# Systematic Toxicological Analysis of Xenobiotics – Comparison of the results obtained on patient samples using immunoassays, LC/DAD and LC/MS methods

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**AIMS:** Both immunoassay and chromatography based methods are used in the toxicology laboratory of the Lille Hospital for the xenobiotics routine screening. The choice of the method depends not only on the analytical performances and possibilities, but also on the ease of use. This parameter is especially important for emergency diagnostic during the night, when skilled analytical chemists are not available. In this situation, immunoassays are often used to provide preliminary information. However, this approach is targeted and limited to some classes of molecules in specific matrices. LC separation coupled either to UV diode array detection or to MS detection permits the analysis of a much broader range of analytes in all biological matrices after extraction. We have developed a mass spectra library for use in conjunction with LC/MS. Here we present the results of a comparative study of these three approaches performed on samples coming from the intensive care units.

**METHOD:**

*Immunoassay:* Tests were used on plasma for intoxication diagnostic of phenobarbital, tricyclic antidepressants, benzodiazepines, or in urine samples for barbiturates, benzodiazepines, opiates, cocaine, amphetamines and cannabinoids.

*LC/DAD:* The identification of the xenobiotics is based on their retention time and UV spectra obtained in pre-defined conditions. The UV spectra are compared to a spectra library of 1072 compounds and metabolites, using the compound retention times as a filter for the library search.

*LC/MS:* The identification of the compounds is based on their MS spectra. Ionization is made in positive and/or negative electrospray, and spectra are generated by fragmentation in the source at various cone voltages. Up to twelve spectra are generated and kept in the library for each compound, depending on the ionization mode and cone voltage values. Only information rich spectra are entered in the library. At present, the MS library counts more than 2500 spectra corresponding to more than 500 compounds. The Total Ion Chromatograms corresponding to the various acquisition cone voltages, are automatically deconvoluted and library searched using the Waters ChromaLynxTM software. Retention times can also be used as library search filter.

Chromatographic methods are used after sample preparation by liquid-liquid extraction in acidic and basic conditions.

For LC/DAD and LC/MS analysis, the same sample vial was analyzed successively on both instruments. This comparison was performed on 33 serum and 30 urine samples, as sent by the emergency department of the hospital. For urine samples, analyses were done with and without beta glucuronidase treatment. Only results reported automatically by the software have been considered.

**RESULTS AND DISCUSSION:** Each method has known advantages and limitations. Immunoassays are available only for some drug categories and therefore constitute a targeted approach. We will show a number of cases where immunoassays did not provide the identification of toxicant, or simply gave the molecule class, while chromatographic methods identify the xenobiotics. For example intoxication cases involving zolpidem are not detected by the immunoassays methods,, while detected in our screening approach. In an other intoxication case, the LC/MS library search indicated both diazepam and nordiazepam, plus ceterizine and hydroxyzine, while immunonassays indicated only the presence of benzodiazepines.
Chromatographic based methods have a wider application domain, but can be used only after a suitable sample extraction and clean-up, which is a discriminating step. MS detection might also be prone to ionization suppression, which can limit the sensitivity for some compounds. The clear advantage of chromatography based methods over immunoassays is the ability to identify molecules from other drug classes and therefore to detect polyintoxication cases. Compared to UV diode array detection, MS detection provides additional sensitivity and selectivity. This allows the identification of molecules not detected with UV detection like some benzodiazepines, antidepressants, or meprobamate, as will be shown in multiple real intoxication cases.

**CONCLUSION:** With this study, we confirm that chromatographic techniques have a wider application range than immunoassays, which remain however of high interest due to their ease of use. In all studied samples, MS detection with automatic data deconvolution gave more complete information than UV detection. This LC/MS method is now used in our laboratory for the general unknown screening, as a complement or as a replacement of the other methods. Specific demands, like the determination of drugs of abuse are covered by LC/MS/MS methods, or GC/MS; not shown here.

**KEYWORDS:** *Xenobiotics routine screening, Chromatography, Immunoassay*

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