Fully automated platform for TDM analysis in serum samples

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1. Introduction

Therapeutic drug monitoring of concentrations of drugs in body fluids, usually plasma, can be used during treatment and for diagnostic purposes. For selected drugs (Antiepileptics, Benzodiazepines, Neuroleptics and Tricyclic Antidepressant) therapeutic drug monitoring aims to enhance drug efficacy and reduce toxicity.

Antiepileptics are a class of compounds used for neurological disorder characterized by partial or generalized convulsion and impaired consciousness. Most of Antiepileptics show a pronounced intra and interindividual variability in pharmacokinetic.

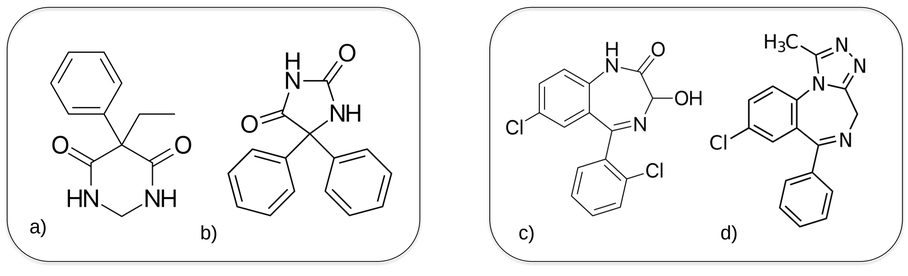
Benzodiazepines are psychoactive drugs used as anxiolytics, sedatives and anticonvulsants. Benzodiazepines share a 5-phenyl-1,3-dihydrobenzo[e][1,4]diazepine nucleus. Despite similar chemical structure the drugs differ in pharmacokinetics and metabolic properties. The quantification of these compounds is primarily performed from serum in order to optimize the drug dosing, to verify consumption compliance and to identify changes in pharmacokinetics.

Tricyclic Antidepressants are a group of psychoactive drugs mainly used for therapy of endogenous depression, anxiety and pain management.

Neuroleptics are a class of psychoactive drugs use in therapy of psychoses, in particular the symptomatic treatment of schizophrenia. All Neuroleptics share the acute onset effect correlates with the current blood level.

For all the previously described drugs the TDM need a to be accomplished by extremely accurate techniques because of their narrow therapeutic range. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) show higher sensitivity and superior specificity compared to immunoassay-based approaches. Moreover LC-MS/MS could easily accomplish multiplexing measurements (simultaneous quantification of several drugs), resulting in relatively high throughput and important cost savings. Some disadvantages such as lack of automation and standardization, the need of qualified staff are currently limiting the use of LC-MS/MS in routine analysis of clinical core labs.

We report the use of fully automated platform for the quantitation of four major drugs classes in serum samples with high throughput, random access capability and without operator sample preparation.



ANTIEPILEPTICS | BENZODIAZEPINES | NEUROLEPTICS | TRICYCLIC ANTIDEPRESSANTS

Figure 1: example of molecules incluse in TDM panel; a) Primidone b) Phenytoin c) Lorazepam d) Alprazolam e) Olanzapine f) Clozapine g) Doxepin h) Imipramine

2. Material and Method

The analysis of Antiepileptics, Benzodiazepines, Neuroleptics and Tricyclic Antidepressant were performed using a fully automatic LCMS preparation Unit (CLAM-2000, Shimadzu, for research use only) online with HPLC-LCMS (NexeraX2 - LCMS8060, Shimadzu) starting from serum samples using the “ClinMass® TDM Kit System” Recipe, (MS9200 for Antiepileptics, MS 9500 for Benzodiazepines, MS9300 for Neuroleptics and MS9100 for Tricyclic Antidepressant). Samples (Reference material: human serum samples), calibrators and Internal Standard mix were loaded onto the CLAM-2000 (refrigerated at 8°C). The treated samples were separated by the analytical column (Recipe, MS9030) for all panels with a binary gradient system (Mobile phase A MS9007, mobile pahse B MS9008) at a different flow rate (Table 1). Quantification was performed using optimized MRM transitions and Internal standard calibration method.

[LC] Nexera X2 System	ANTIEPILEPTICS	BENZODIAZEPINES	NEUROLEPTICS	TCAs
Column Temp. (°C)	40	40	40	40
Time Program	grad. A-B 5.5 min	grad. A-B 7.5 min	grad. A-B 5.5 min	grad. A-B 3.8 min
Injection Vol (µL)	1	2	0.5	0.3
[MS] LCMS-8060				
Ionization	ESI +/-	ESI +	ESI +	ESI +
Nebulizer Gas (L/min)	3	3	3	3
Interface Temp (°C)	300	300	400	300
Desolvation Line (°C)	250	250	225	250
Heat Block Temp (°C)	400	400	400	400
Drying Gas (L/min)	10	10	10	10
Sacn Type	MRM	MRM	MRM	MRM

Table 1 Analytical conditions.

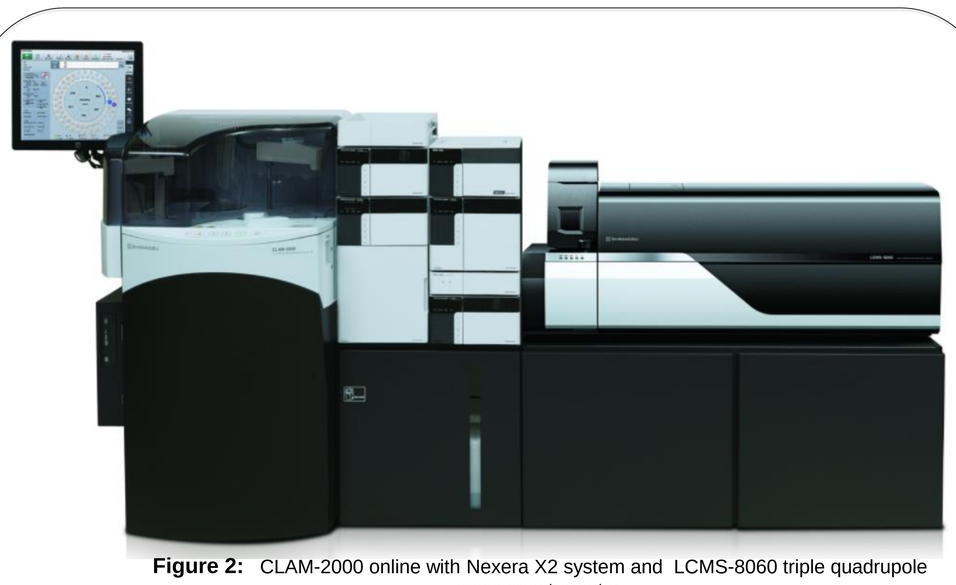


Figure 2: CLAM-2000 online with Nexera X2 system and LCMS-8060 triple quadrupole mass spectrometer.

3. Results

Prior to the LC-MS/MS analysis a sample preparation is carried out in order to remove the sample matrix and to spike the sample with an internal standard. This procedure is time consuming and could be affected by bias caused by the operator due to the liquid transfer steps that are required, moreover it is difficult maintain the traceability of each steps for all the processed samples. Using the CLAM-2000 it was possible to obtain a complete integration of sample preparation steps with the LC-MS/MS quantification.

The samples were loaded onto the CLAM-2000 using disposable microvials or primary testing tubes. The fully automatic preparation/analysis procedure was performed as follows: I) 20 µl of methanol were dispensed in a filtration-collection vial; II) serum sample were added; III) IS mix were added (protein precipitation); IV) shaking and incubation; V) filtration for 0.45 min (deproteinization); the sample was finally transported to the LCMS system without human intervention for the quantification and the results were directly visualized by CLAM-2000 software control.

CLAM-2000 can easily change analytical method and sample preparation procedure by automatically overlap different analysis panels (figure 3). Using this function it was possible to operate in random access mode over a **total of 91 molecules.**

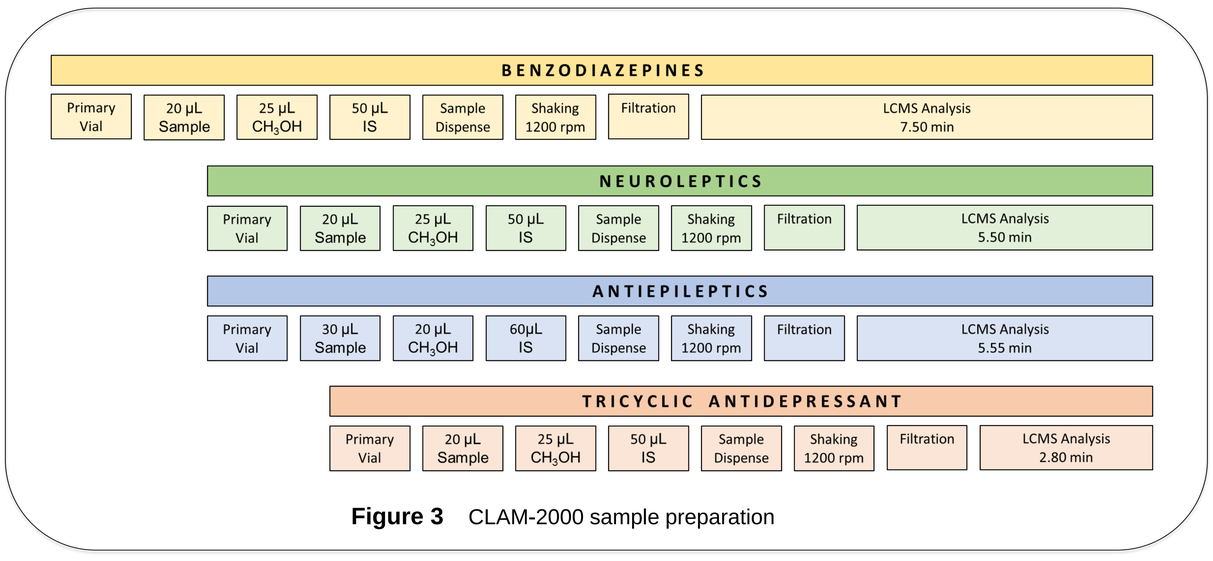


Figure 3 CLAM-2000 sample preparation

The linearity and accuracy of the methods were evaluated using 3 reference serum calibrators levels for each panel (Recipe MS 9213 for Antiepileptics, MS6013 for Benzodiazepines, MS9313 for Neuroleptics, MS9113 for TCAs). For all the analytes linearity and accuracy were within the analytical acceptable range (85%-115%) in all panels analyzed. Furthermore in order to estimate the precision of the method, reference serum samples (Recipe MS9282 for Antiepileptics, MS6082 for Benzodiazepines, MS9382 for Neuroleptics, MS9182 for TCAs) spanning from low concentration level to high concentration level were analyzed several times (6 replicates). For all analytes the CV% values were within acceptable analytical ranges. The same experiment was repeated for 4 non-consecutive days in order to estimate the inter-day precision.

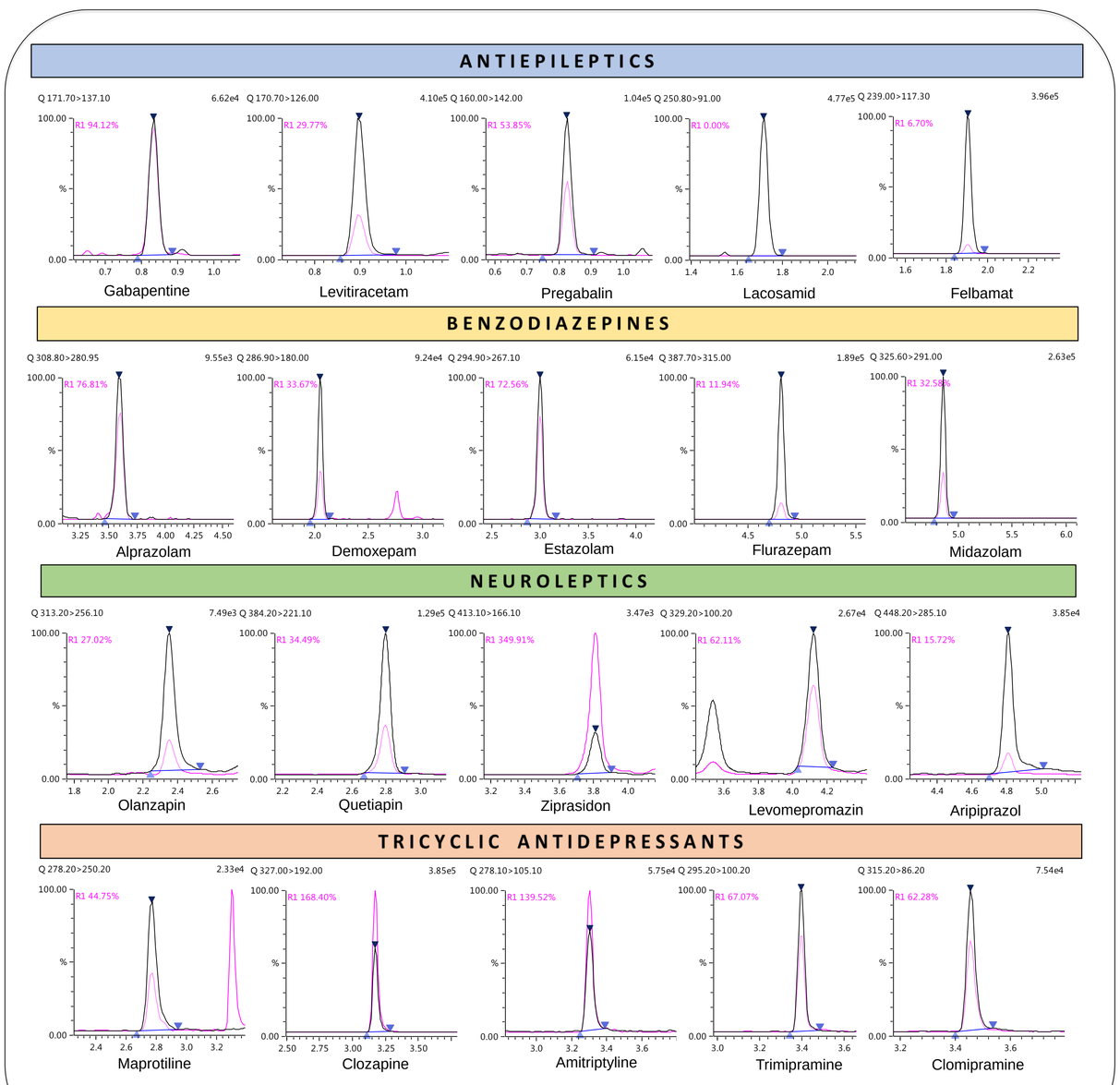


Figure 5: example of chromatograms of Antiepilepics, Benzodiazepines, Neuroleptics and Tricyclic Antidepressants at LLOQ.

4. Conclusions

- Fully Automated sample preparation procedure resulted suitable for the quantitation of different panels of molecules (Antiepileptics, Benzodiazepines, Neuroleptics, Tricyclic Antidepressants) by elimination of all manual preparation steps.
- The automation of the method increases the analytical performance, reduces the risk for human operators and due to the reduced reagent consumption, reduces also the cost of the analysis.
- The possibility to run all the samples in random access mode using CLAM-2000 reduce sample preparation time and increase the flexibility of the platform.