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A LC-MS method for the measurement of 245 compounds of interest in toxicology with a fully-automated sample preparation

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Overview

- A **fully automated quantitative** screening method was developed measuring 245 illicit and medical compounds from blood, plasma and urine.
- The method was **validated** according the requirements of ISO 15189.
- **A robustness study** showed that calibration curves prepared up to one month old provided an uncertainty of less than 20% for more than 80% of the compounds.
- **Comparison** to other quantitative methods was made using 188 patient samples providing a regression correlation R^2 = 0.85 (n=312 measured concentrations).

Introduction

For screening of drugs two steps are needed: an analysis for identification and another for the measurement of detected compounds. At both stages, manual multiple-step extraction procedures are usually used before LC-MS analysis. We propose a solution: (i) using a **fully-automated extraction** procedure directly coupled to the LC-MS system, with **no human intervention**; (ii) that **simultaneously identifies and quantitates** compounds of interest for toxicological application, based on an **MRM spectrum mode** (MRM-SpM) method where up to 15 MRM transitions per compound are followed. Ion intensities from each transition are used to construct an MRM spectrum that could be used to search against registered library spectra. As conventional MRM method, MRM transition enable quantification of the compound.

Methods

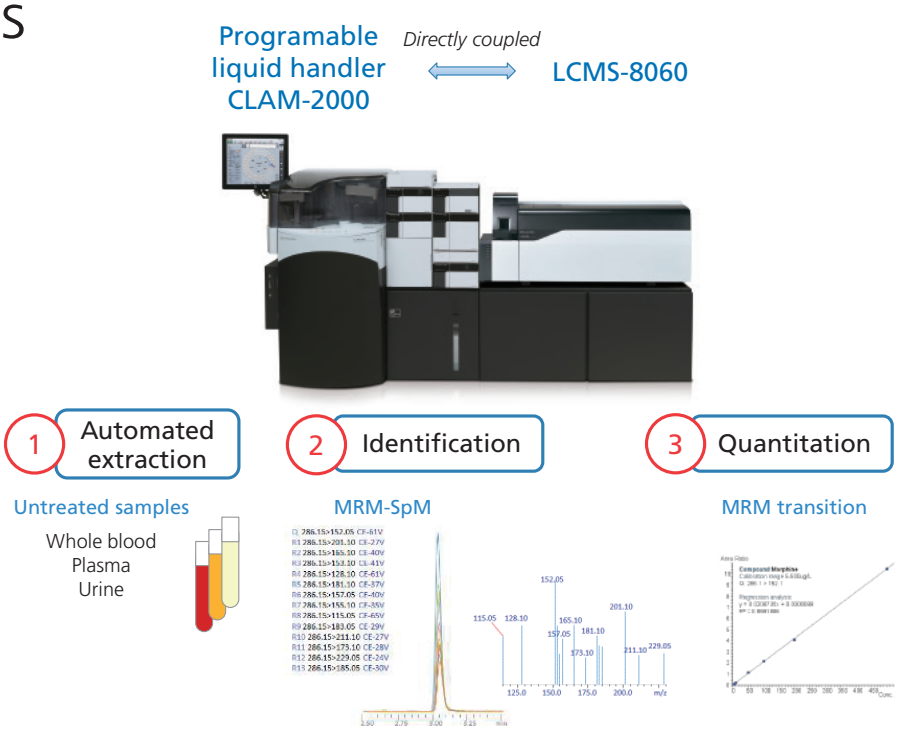

Figure 1: Automated sample preparation for clinical sample analysis fully integrated with LC-MS/MS detection and quantitation.

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Results

The analytes selected for targeted screening were based on the likelihood of encountering the compound in a clinical scenario. 245 compounds were chosen and summarized in Figure 2.

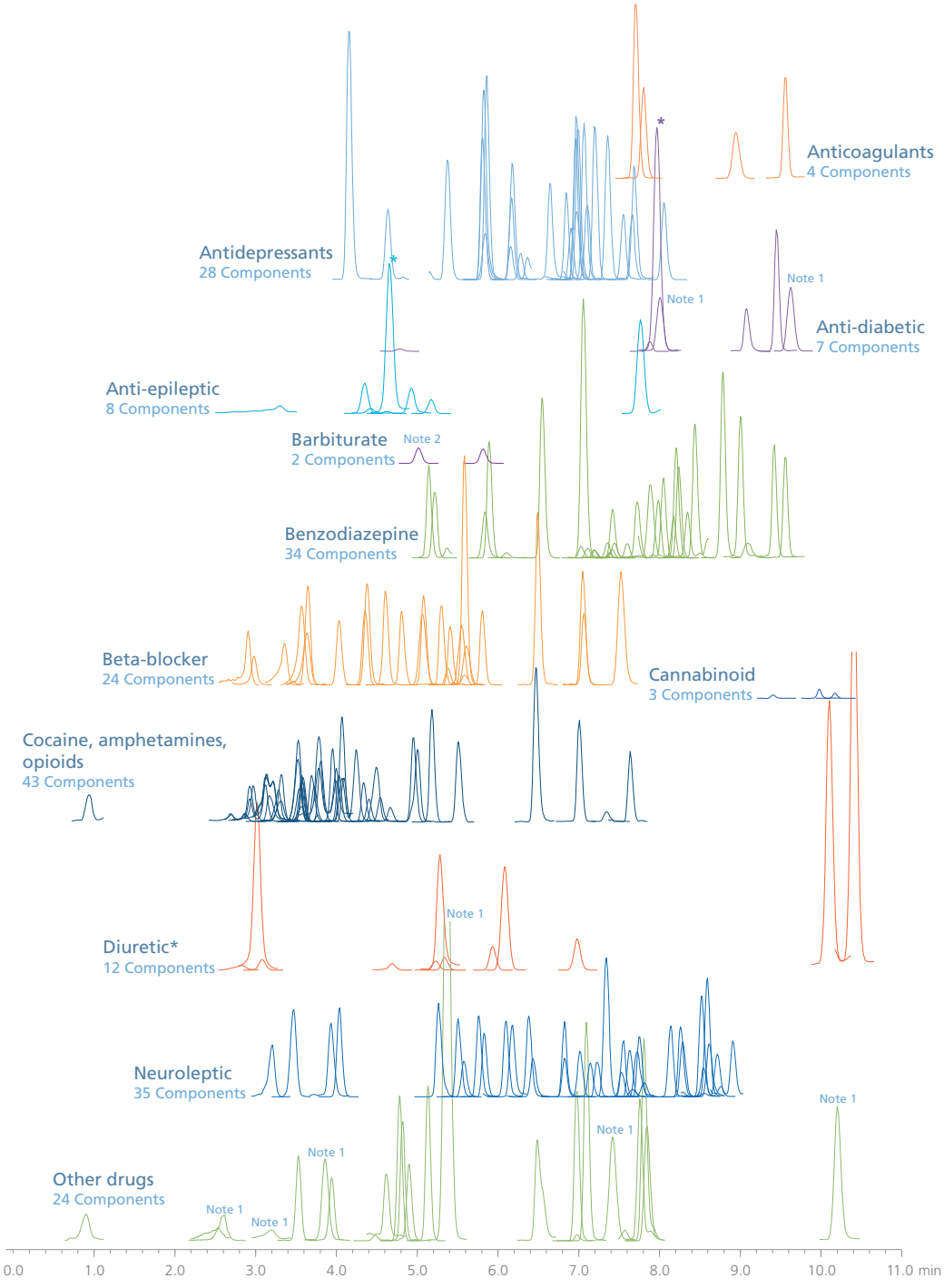

Figure 2. MRM chromatograms of compounds extracted from whole blood at a concentration of 5ng/mL (chromatograms shown at this concentration with the exception of 50ppb[1] and 1ppm[2]). All compounds were identified using MRM Spectrum mode and library searching. Concentration 5ppb spiked in whole blood with exception to *50ppb, #1ppm (scale normalised to 1250000 for all chromatograms).

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Validation and robustness study

The method was fully validated according the requirements of ISO 15189. In this work, ultra-fast data acquisitions enabled methods to be set up to measure 245 compounds with an average of 12 MRMs per target compound and 2 MRMs per ISTD. Even with such high data density, quantitative data quality and library identification was not compromised (for example desmethylflunitrazepam and N-desmethylclobazam; figure 3).

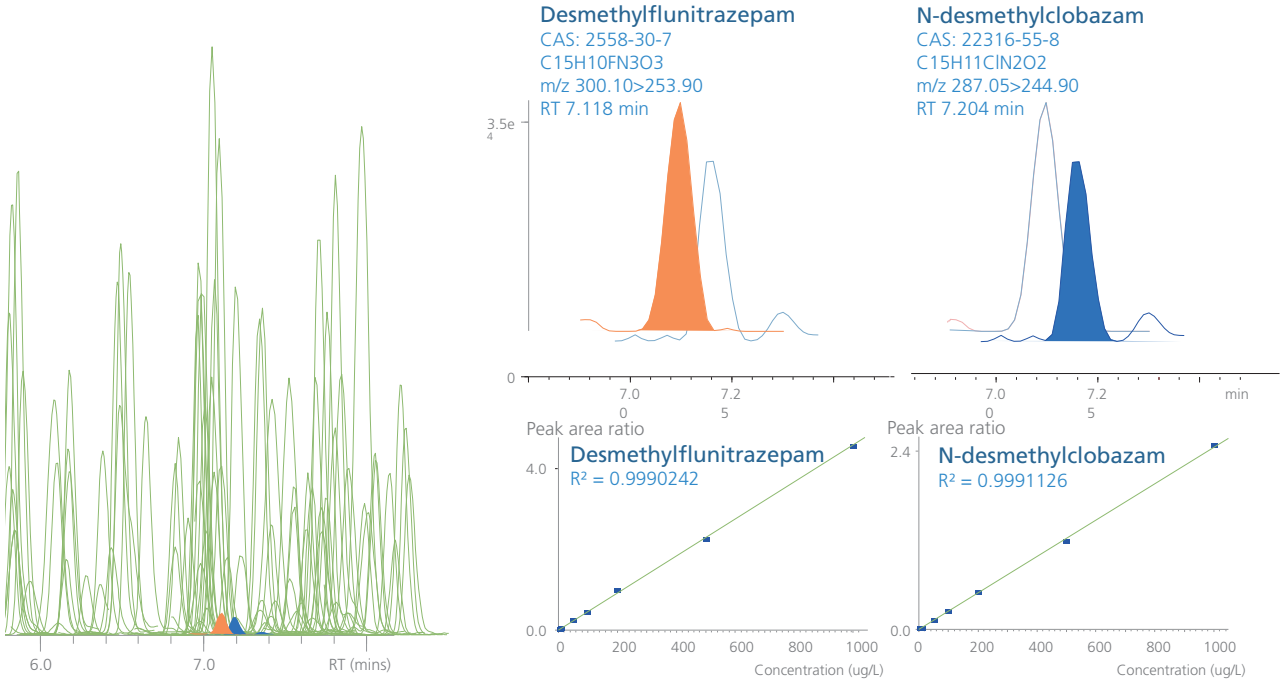

Figure 3. Desmethylflunitrazepam and N-desmethylclobazam elute at 7.12 and 7.202 mins. Despite a time window of monitoring over 400 simultaneous MRMs the compounds resulted in linear calibration curves (R^2>0.999)

A robustness study was performed to evaluate how long acceptable quantitative accuracy could be provided by historic calibration curve data files. The study was based on a reference mix of 23 compounds. Quality control samples (LOQ) sample data was first quantified using calibration standards prepared on the same day and then re-processed using historic calibration standard data files from up to 4 weeks old (n=9). Accuracy variation between 85-115% was obtained for 74% of the compounds and the maximum CV was 18.3% (Table 1).

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Table 1. Accuracy and CV obtained for the reference mix during a one month period

	Accuracy									CV
	Calibration 1	Calibration 2	Calibration 3	Calibration 4	Calibration 5	Calibration 6	Calibration 7	Calibration 8	Calibration 9	
	Tuesday old	1 day old	4 days old	6 days old	10 days old	16 days old	22 days old	24 days old	30 days old	
Amiodarone	83.89	83.95	70.85	63.2	81.93	89.88	95.1	87.2	91.04	**12.19**
Amphetamine	72.48	71.8	64.88	84.3	70.11	92.01	71.04	103.1	97.84	**17.16**
Apixaban	119.72	123.91	134	101.3	107.02	132.41	112.76	118.2	116.43	**9.10**
Aripiprazole	86.56	99.26	93.92	69.32	90.4	99.9	78.77	115.8	100.65	**14.65**
Atenolol	99.23	135.32	122.54	69.52	105.92	116.28	105.21	87.9	104.41	**18.23**
Benzoylecgonine	115.93	122.87	121.88	84.05	106.95	126.33	112.14	117.5	104.56	**11.45**
Buprenorphine	64.4	116.43	81.03	67.82	79.16	90.25	80.4	71.7	96.1	**19.38**
Citalopram	83.17	71.12	80.66	81.21	82.29	97.66	62.55	115.5	96.93	**18.31**
Haloperidol	107.82	119.18	98.28	95.39	95.54	99.91	89.22	111.7	125.92	**11.66**
Ketamine	111.71	101.55	110.7	114.77	111.27	126.97	117.37	89.8	111.97	**9.31**
Lidocaine	90.07	99.78	86.25	60.14	84.68	96.2	80.48	94.2	94.73	**13.64**
Metformine	83.38	93.66	87.58	65.05	79.89	93.06	125.99	102.2	95.37	**18.23**
Morphine	83.47	104.25	89.27	77.29	89.27	115.77	93.21	95	83.93	**12.65**
Nordiazepam	115.19	139.19	135.56	108.89	112.5	95.8	122.79	116	118.53	**11.18**
Phenobarbital	100.39	106.85	119.63	87.18	109.51	106.97	105.09	100.8	98.71	**8.52**
Salicylic acid	63.42	75.88	80.97	90.54	60.87	87.66	84.5	94	95.64	**15.46**
THC	94.49	106.92	107.77	80.93	108.88	134.55	112.19	120.7	98.62	**14.31**
THC-COOH	88.71	71.28	107.2	102.1	105.16	100.09	90.02	106.8	123.5	**14.79**
Tramadol	129.65	142.5	144.44	108.84	127.23	108.84	100.41	131.3	98.98	**14.38**
Verapamil	98.7	129.75	107.26	83.28	115.14	102.36	91.07	102.1	93.78	**13.41**
Warfarin	117.98	119.32	121.8	101.31	116.05	128.62	107.96	125.8	107.53	**7.79**
Zonisamide	73.25	83.61	87.83	69.57	84.85	94.27	84.2	94.8	92.78	**10.43**

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Application of the whole procedure to real samples

The whole automated sample preparation and LC-MS/MS analysis was tested with 188 patient samples (134 plasma, 11 whole blood and 43 urine) comparing quantitative results from the newly developed method and pre-existing validated methods, routinely used in our lab (i.e., LC-MS/MS methods dedicated to one class of compounds). The developed method was measured by MRM Spectrum mode (up to 15 MRMs per compound) whereas the others analyses measured samples using a conventional MRM method (2-3 MRMs per compound). When a compound was positively detected with a reference method, it was also detected by the new method. Seventy eight different compounds were detected in the patient samples corresponding to a total of 312 data points in a regression analysis that reported a good agreement (R^2 0.85, slope 0.81). A summary of this study for plasma samples are summarized in Figure 4.

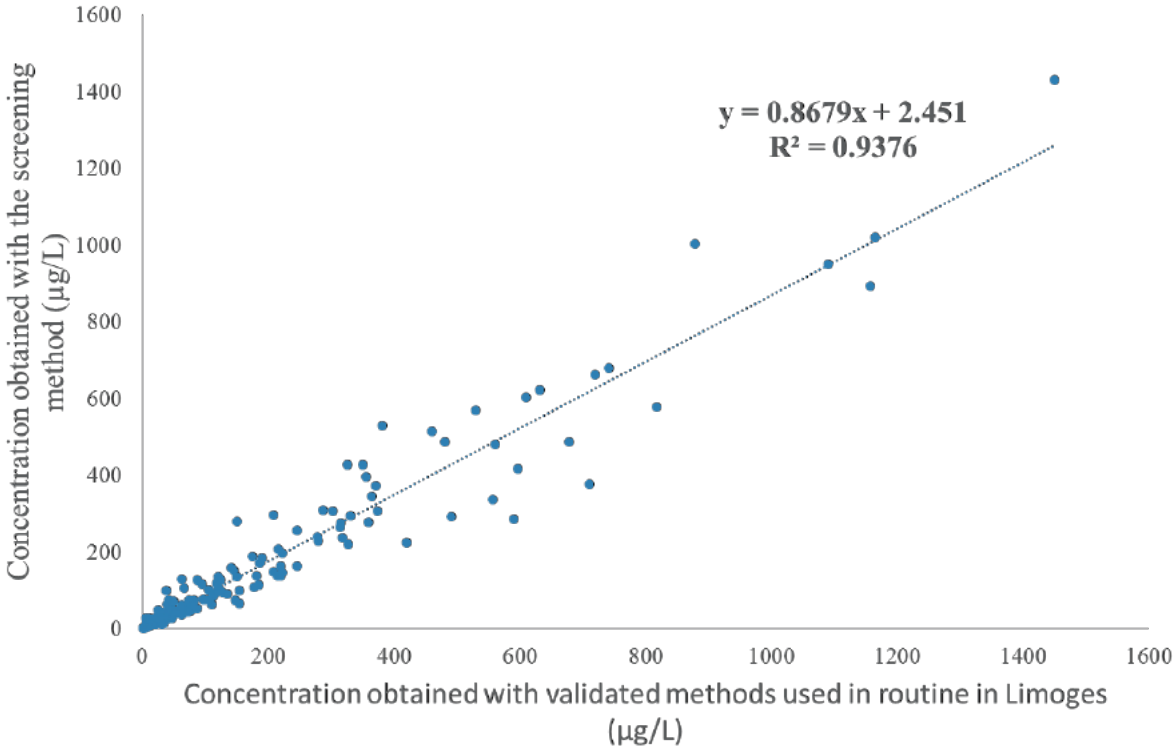

Figure 4. Analysis of 134 plasma samples with reference methods and the newly procedure

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Conclusions

We have completed quantitative screening using a LC-MS/MS coupled to an automated sample preparation system for a panel of 245 compounds of interest for clinical and forensic application. The most frequently detected compound in clinical toxicology are covered and results show good capacity of this solution to provide high confidence in compound identification and good accuracy. The automation of the procedure drastically decreases the time of analysis enabling to work on other tasks while the system perform the analysis.

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