

Quantification of recent antidepressants from micro whole blood samples collected on filter-paper by gas chromatography coupled with tandem mass spectrometry

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Introduction

Since 1980, important antidepressants were developed including fluoxetine, paroxetine and reboxetine. Even if these new compounds have fewer undesirable effects, they are frequently used in deliberate self-poisoning and they can lead to major intoxications. Thus, antidepressants analysis is required in many fields such as therapeutic drug monitoring (TDM) or forensic toxicology.

In the 60 decade, an alternative sampling procedure appeared allowing to collect blood samples on filter paper. Called dried blood spots (DBS), this procedure offers many advantages compared to classic venous sampling.

Due to the small sample volume (i.e. 10 µL), a sensitive analytical method is required and an interesting approach is the use of negative-ion chemical ionization (NICI) known for its high sensitivity and selectivity. However, NICI requires a derivatization step to improve the electro-affinity of the analytes, but this step is often tedious and time consuming. This work was particularly focusing on the optimisation of the antidepressant's derivatization which was combined in a single step with extraction of DBS.

This poster describes a new efficient method for the quantitative monitoring of 3 antidepressants and one phase I metabolite in whole blood by combining DBS sampling procedure with an accelerated gas chromatography (GC) separation coupled to NICI-MS/MS detection.

Experiment

• DBS sample pretreatment

Paroxetine, reboxetine, fluoxetine and norfluoxetine were analysed from 10 µL of whole blood spotted on filter-paper as described on figure 1.

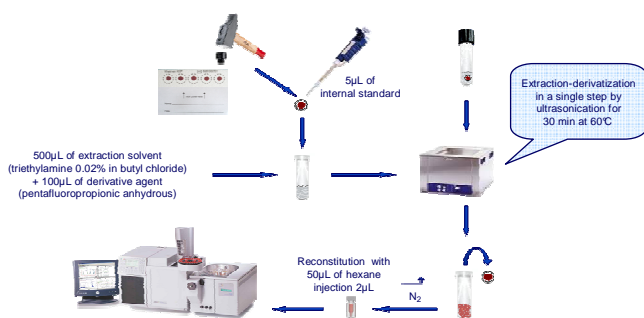


Figure 1. DBS sample preparation method combines an extractive-derivative single step with a GC-QqQ-MS/MS system

• GC-NICI-MS/MS conditions

Substances were separated using a GC system with a fused-silica capillary column (DB-5MS, 15 m x 0.25 mm i.d., film thickness 0.25 µm) and high-purity helium 50 (99.999%) with a constant flow of 1 mL/min. The injector temperature was set at 300°C and splitless injection was employed. The initial column temperature was set at 105°C for 1 min, increased to 300°C at 70°C/min, and held for 1.22 min for a total analysis time of 5 min.

Mass spectrometric detection was performed with a QqQ system in selected reaction monitoring mode (SRM) after NICI ionization. Methane 55 (99.9995%) was used as reagent gas at a pressure contained between 8 and 8.5 Torr. Transfer line, manifold and ionization source were operated at 275, 40 and 150°C respectively.

Results

Analytical method was validated according to a general strategy based on the guidelines of the "Société Française des Sciences et des Techniques Pharmaceutiques" (SFSTP).

The limits of quantification (LOQ) were fixed at 1 ng/mL for fluoxetine and its metabolite and 20 ng/mL for reboxetine and paroxetine. The limit of detection (LOD) was found to 20 pg/mL for all analytes, and the repeatability was less than 15% for all concentrations. (See table 1).

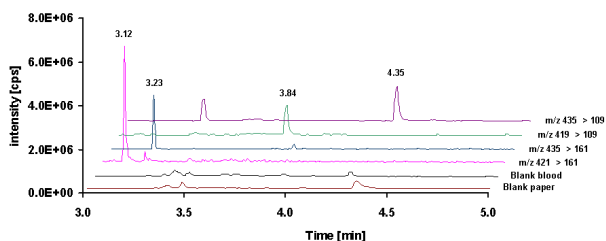


Figure 2. DBS sample spiked with 20 ng/mL of each antidepressant

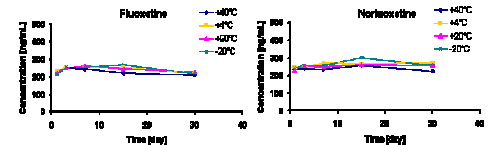
The figure 2 shows a typical chromatogram at the low concentration of quality control. Selectivity was also tested with a blank blood and blank paper and none peak were found at the retention time of the antidepressants.

Antidepressants	Nominal conc. (ng/mL)	Founded conc. (ng/mL)	Relative bias (%)	Repeatability (R.S.D. %)	Intermediate precision (R.S.D. %)
Norfluoxetine 421 > 161	1	0.99	-1.1	6.0	6.0
	20	21.3	6.5	4.6	4.6
	250	264	5.8	6.2	9.1
	500	497	-0.6	9.7	11.9
Fluoxetine 435 > 161	1	0.99	-1.3	5.5	8.2
	20	19.9	-0.5	8.5	8.5
	250	272	8.7	6.2	6.2
	500	551	10.1	2.5	7.6
Reboxetine 419 > 109	20	21.6	7.7	7.6	10.1
	40	43.7	9.1	5.1	8.3
	250	256	3.1	8.3	8.3
	500	552	10.3	6.7	8.4
Paroxetine 435 > 109	20	21.4	7.0	6.5	9.8
	40	38.5	-3.8	5.8	5.8
	250	261	4.4	4.8	6.4
	500	556	11.2	5.1	7.4

Table 1. Validation data

Short term stability

In order to test the short term stability of the antidepressants on filter paper, three batches of antidepressant-free whole blood were spiked with all analytes at 250 ng/mL.



Then, DBS samples were subjected to storage at different temperatures: -20°C, 4°C, 20°C, and 40°C. Stability study was carried out over one month with time points of 1, 3, 7, 15 and 30 days.

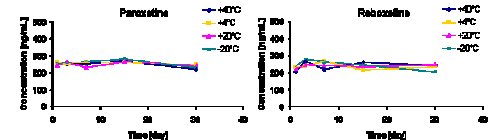


Figure 3. DBS stability for the antidepressants under different storage conditions

This figure 3 shows good stability of fluoxetine, norfluoxetine, paroxetine and reboxetine in dried blood spots over 30 days since all DBS tested gave concentrations between 85 and 115% of the initial concentration.

Pharmacokinetic of fluoxetine

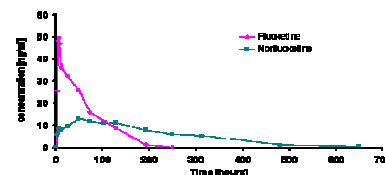
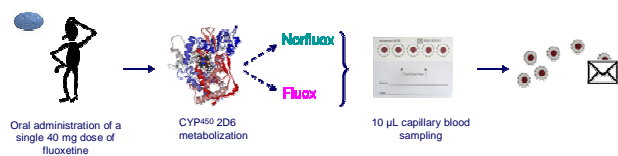


Figure 4. Concentration vs. time profile of fluoxetine and norfluoxetine in whole blood of one volunteer after receiving 40 mg fluoxetine

Conclusion

This poster presents a validated DBS method which combines an extractive-derivative single step with a fast and sensitive GC-NICI-MS/MS technique for the quantification of antidepressants (fluoxetine, paroxetine and reboxetine as model compounds).

The procedure, with its easy to use sample collection and a micro volume of blood (i.e. 10 µL), offers a patient friendly tool in many analytical fields such as treatment adherence control, therapeutic drug monitoring, toxicological analyses, or when multiple samples are required such as pharmacokinetic studies.

Moreover, short-term stability showed no significant difference between the four temperatures tested. Thus, DBS sampling process provides a powerful alternative tool in terms of storage and shipment compared to classic venipuncture.