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Short communication

# High-performance liquid chromatographic method with diode array detection to identify and quantify atypical antipsychotics and haloperidol in plasma after overdose

K. Titier*, S. Bouchet, F. Péhourcq, N. Moore, M. Molimard

*Department of Clinical Pharmacology and Toxicology, Pellegrin Hospital and University Victor Segalen, Place Amélie Raba-Léon, 33076 Bordeaux, France*

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**Abstract**

For toxicological purposes, an HPLC assay was developed for the simultaneous determination of haloperidol and atypical antipsychotics (risperidone, 9-hydroxyrisperidone, olanzapine, clozapine) in human plasma. After a double-step liquid–liquid extraction, compounds were separated on a $C_8$ column eluted with a gradient of acetonitrile and phosphate buffer 50 m*M* pH 3.8. A sequential ultraviolet detection was used (260, 280 and 240 nm). Calibration curves were linear in the range 10–1000 ng/ml. The limits of quantification were 5 ng/ml for all drugs. Average accuracy at four concentrations ranged from 93 to 109%. Both inter- and intra-day variation coefficients were lower than 11% for all drugs. This simple and rapid method (run time<15 min) is currently used for poison management.


*Keywords:* Haloperidol; Risperidone; 9-Hydroxyrisperidone; Olanzapine; Clozapine

## 1. Introduction

Atypical antipsychotics (olanzapine, clozapine, risperidone and its active metabolite, 9-hydroxyrisperidone) and haloperidol make up a large market share of the treatment of schizophrenia and other psychoses. Suicide attempts are very frequent in this user population, and several intoxications involving these compounds have already been published [1–4]. Neuroleptic toxicity is especially associated with myocarditis, arrhythmia [5–7] and sudden death [8]. This justifies the development of a screening method for clinical or forensic toxicology.

Determination of some of these drugs using ultraviolet spectrometry [9–12], electrochemical detection [10,13] or mass spectrometry [14–19] has been described. However, none of these methods allows the rapid quantification and identification of haloperidol and atypical antipsychotics in a single run.

The aim of this study was to establish a simple, rapid and accurate high-performance liquid chromatographic (HPLC) method for the identification

*Corresponding author. Tel.: +33-5-5679-5991; fax: +33-5-5624-5889.
*E-mail address:* karine.titier@pharmaco.u-bordeaux2.fr (K. Titier).

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and measurement of haloperidol and atypical antipsychotics in plasma, after overdose. The assay requires a small sample volume, involves a liquid extraction with a specific internal standard and a short chromatographic run.

## 2. Experimental

### 2.1. Chemicals

Haloperidol (HLP), risperidone (RSP), 9-hydroxyrisperidone (OH-RSP) and internal standard (I.S., methylrisperidone), were generously donated by Janssen Foundation (Beerse, Belgium). Olanzapine (OLZ) and clozapine (CLZ) were kindly donated by Lundbeck (Copenhagen, Denmark). All reagents used for the assay were of HPLC or analytical grade. Water was deionized and purified by a Milli-Q system (Millipore, Bedford, MA, USA). Heparinized healthy human volunteers plasma was purchased from Etablissement de Transfusion Sanguine d'Aquitaine (ETSA, Bordeaux, France).

### 2.2. Standard solutions

Stock solutions of RSP, OH-RSP, CLZ, HLP, OLZ and I.S. were prepared at a concentration of 1 mg/ml in methanol and stored at −20 °C. They were stable for at least 3 months. The internal standard stock solution was diluted daily in double distilled water to yield a 3 μg/ml working solution.

Plasma standards containing known amounts of drugs were prepared by spiking plasma to yield concentrations of 10, 20, 50, 100, 500, 800 and 1000 ng/ml. Plasma quality controls spiked with 5, 10, 30, 200 and 900 ng/ml were prepared to test the accuracy and precision of the method.

### 2.3. Equipment

The HPLC unit consisted of an Alliance® 2690 separation module and a 996 photodiode array detector controlled by Millenium$^{32}$ version 3.2 software (Waters, Milford, MA, USA).

Separations were carried out at 30 °C on a Symmetry $C_8$ 250×4.6 mm (Waters). The mobile phase consisted of a gradient of phosphate buffer 50 m*M* pH 3.8–acetonitrile, increasing linearity from 15 to 35% acetonitrile over 5 min, and to 63% for the next 6 min. The system was then stabilized for an additional 4 min with the initial conditions. Phosphate buffer 50 m*M* pH 3.8 for the mobile phase was prepared by dissolving of 6 g of $NaH_2PO_4$ in 1 liter double distilled water and adjusting to pH 3.8 with orthophosphoric acid 10%. The mobile phase was filtered through a 0.45-μm filter (Sartorius, Goettingen, Germany). The total run time of the analysis was 15 min at a flow-rate of 1.5 ml/min.

The UV-detector program consisted of a 5-min sequence set at 260 nm for OLZ acquisition, 2.2-min sequence set at 280 nm for RSP and OH-RSP acquisition followed by a sequence set at 240 nm for I.S., CLZ and HLP acquisition.

Simultaneous acquisition from 210 to 350 nm was carried out for the identification of unknown compounds. Standard solutions were prepared at a concentration of 100 μg/ml in methanol and injected into the HPLC unit to determine the reference ultraviolet absorption spectrum of each compound. Then, unknown compounds were identified by comparison of their spectra to the references.

### 2.4. Sample preparation

To 500 μl of plasma was added 20 μl of I.S. (3 μg/ml) and 100 μl of sodium hydroxide 2 N. The mixture was extracted in 7 ml of hexane–isoamyl alcohol (99:1, v/v) by horizontal shaking during 15 min. After centrifugation (10 min, 5000 rpm), 200 μl of HCl 0.05 N was added to the organic layer. The mixture was shaken for 10 min and centrifuged (10 min at 5000 rpm) and 100 μl of aqueous phase was injected into the HPLC unit.

### 2.5. Assay validation procedures

#### 2.5.1. Recovery

Extraction recoveries from human plasma were determined by comparison of HPLC responses from extracted samples containing known amounts of each drug (30, 200, 600 ng/ml, $n=3$) to those from unextracted and directly injected standards, spiked with the same amounts.

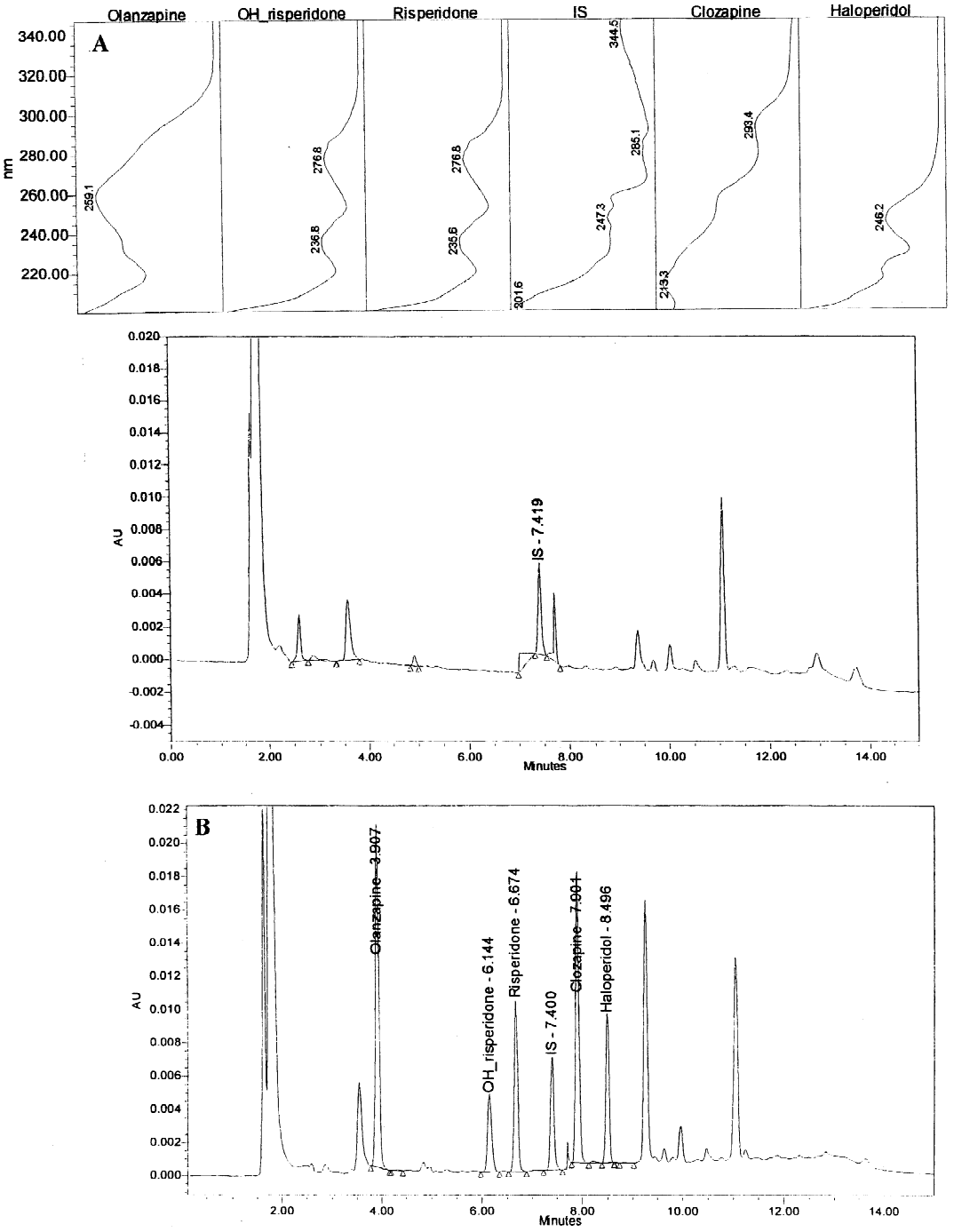


Fig. 1. Chromatograms and ultraviolet absorption spectra obtained from blank plasma (A) and from a sample spiked with 200 ng/ml (B) of olanzapine, hydroxyrisperidone (OH-risperidone), risperidone, clozapine and haloperidol. I.S., internal standard.

*2.5.2. Linearity*

A least squares linear regression was used to calculate the equation relating the peak-area ratio between drug and internal standard and the concentration of five drugs. For all drugs, a weighted regression was used (weighting factor=$1/y^2$, where $y$=area ratio).

*2.5.3. Precision*

The precision of the developed method was determined by analysis of five quality control samples containing 5, 10, 30, 200 and 900 ng/ml of each drug. Intra-day variation of the assay was assessed by injecting six quality control samples at each concentration on the same day. Inter-day variation was assessed by injecting a further six samples of each concentration on three subsequent days. Subsequently, the mean of each set of the concentrations and the percent deviation of the quality control samples were calculated.

*2.5.4. Accuracy*

Accuracy was measured as the % difference from theoretical according to the equation:

$$\text{Accuracy (\%)} = \left( \frac{\text{concentration}_{\text{measured}}}{\text{concentration}_{\text{theoretical}}} \right) \times 100$$

## 3. Results and discussion

### *3.1. Chromatography*

Representative chromatograms of a blank plasma and a plasma spiked with 200 ng/ml of the five drugs are presented in Fig. 1.

Table 1
Intra-day and inter-day precision and accuracy data (mean±SD)

| Drug | Added concentration (ng/ml) | Intra-day ($n$=6) | | Inter-day ($n$=24) | | |
|---|---|---|---|---|---|---|
| | | Observed concentration (ng/ml) | CV% | Observed concentration (ng/ml) | CV% | Accuracy% |
| Olanzapine | 5 | 4.0±0.3 | 6.8 | 4.9±0.9 | 19.0 | 97.0 |
| | 10 | 8.9±0.7 | 7.4 | 9.3±0.8 | 8.2 | 93.4 |
| | 30 | 26.9±1.8 | 6.7 | 28.3±3.0 | 10.6 | 94.5 |
| | 200 | 196.2±5.9 | 3.0 | 199.3±9.2 | 4.6 | 99.7 |
| | 900 | 895.0±31.8 | 3.6 | 890.2±78.8 | 8.9 | 98.9 |
| Risperidone | 5 | 3.9±0.7 | 19.1 | 5.0±0.7 | 15.0 | 99.1 |
| | 10 | 10.6±0.8 | 7.2 | 9.9±0.8 | 8.1 | 98.8 |
| | 30 | 30.6±0.9 | 2.9 | 32.7±2.7 | 8.4 | 108.9 |
| | 200 | 211.3±13.0 | 6.1 | 202.5±12.1 | 6.0 | 101.2 |
| | 900 | 912.2±30.8 | 3.4 | 902.7±40.6 | 4.5 | 100.3 |
| Hydroxy-risperidone | 5 | 4.6±0.3 | 6.6 | 4.9±0.7 | 14.3 | 98.3 |
| | 10 | 10.7±1.1 | 10.5 | 10.0±1.1 | 11.2 | 100.4 |
| | 30 | 29.7±1.9 | 6.2 | 32.4±2.9 | 8.8 | 108.0 |
| | 200 | 214.9±13.6 | 6.3 | 206.2±12.5 | 6.1 | 103.1 |
| | 900 | 901.7±27.9 | 3.1 | 906.2±38.3 | 4.2 | 100.7 |
| Clozapine | 5 | 5.6±0.3 | 5.0 | 5.5±0.5 | 9.5 | 110.2 |
| | 10 | 8.7±0.4 | 5.0 | 9.4±0.9 | 9.5 | 93.8 |
| | 30 | 28.2±0.9 | 3.3 | 32.2±3.0 | 9.2 | 107.3 |
| | 200 | 208.3±9.0 | 4.3 | 205.2±8.1 | 3.9 | 102.6 |
| | 900 | 902.6±39.0 | 4.3 | 890.9±37.0 | 4.2 | 99.0 |
| Haloperidol | 5 | 3.8±1.1 | 3.2 | 4.8±0.8 | 17.5 | 95.2 |
| | 10 | 10.2±0.8 | 7.4 | 9.8±1.0 | 10.6 | 97.6 |
| | 30 | 29.2±1.5 | 5.2 | 32.3±2.5 | 7.8 | 107.7 |
| | 200 | 217.5±15.4 | 7.1 | 209.3±12.3 | 5.9 | 104.6 |
| | 900 | 920.8±38.0 | 3.5 | 897.9±36.1 | 4.5 | 99.8 |

There was no interfering peak in drug-free plasma at the retention times of interest. Under the described chromatographic conditions, retention times were 4.0, 6.2, 6.7, 7.4, 8.0 and 8.5 min for olanzapine, risperidone, hydroxyrisperidone, internal standard, clozapine and haloperidol, respectively.

Measurement of the ultraviolet absorption spectrum of the five antipsychotics and internal standard showed the peak absorbances (Fig. 1). To avoid interferences and maximize the sensitivity of the assay, we chose acquisition wavelengths of 280 nm for risperidone and hydroxyrisperidone, 260 nm for olanzapine acquisition and 240 nm for clozapine and haloperidol.

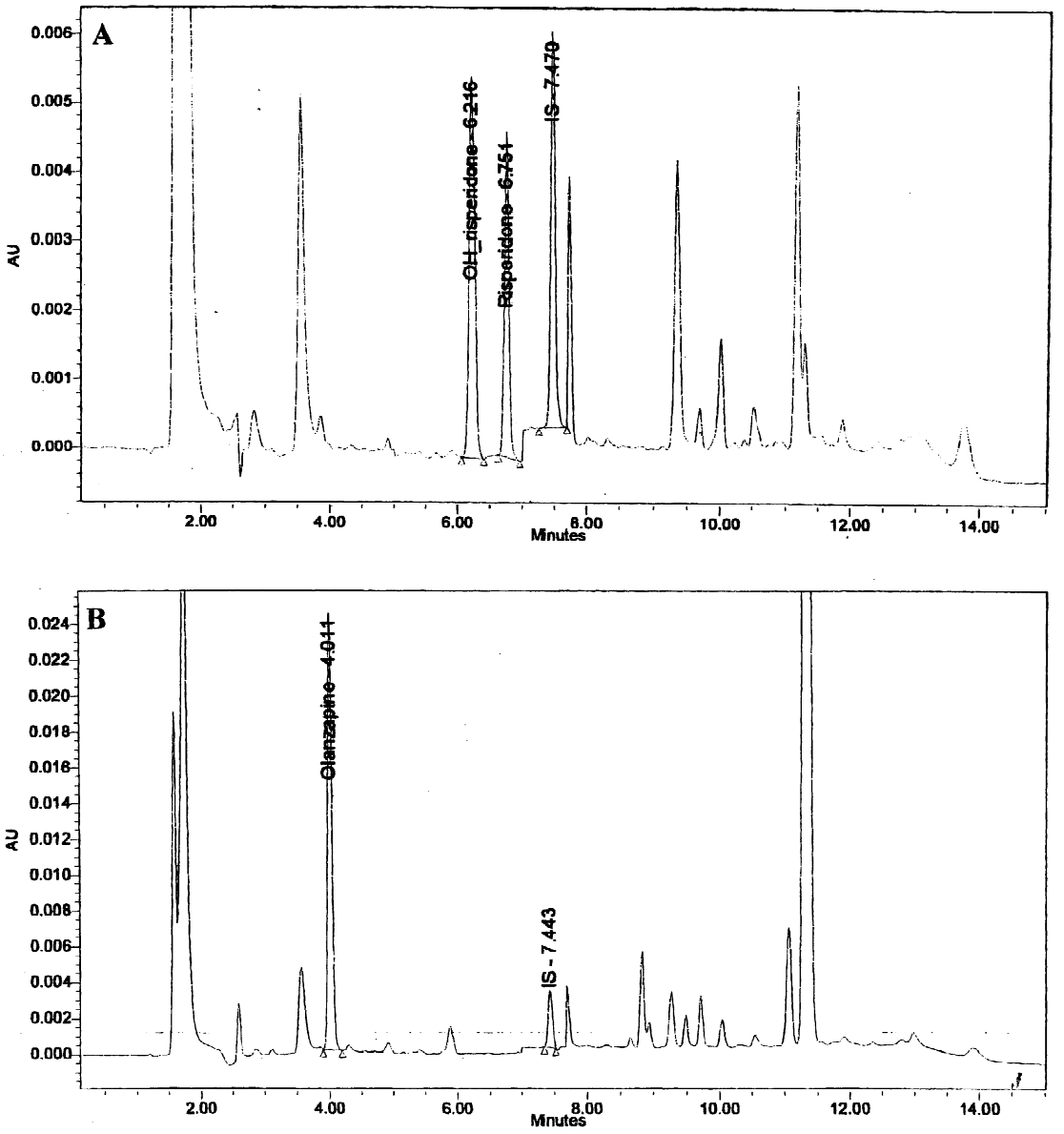


Fig. 2. Chromatograms from patients who had ingested risperidone (A; injection volume 100 μl) and olanzapine (B; injection volume 80 μl). OH-risperidone, hydroxyrisperidone; I.S., internal standard.

### 3.2. Linearity, accuracy and precision

Calibration curves were linear in the range 10–1000 ng/ml ($r^2 > 0.997$) with a slope variation coefficient ($n = 4$) lower than 10%.

The results obtained for precision and accuracy are presented in Table 1 and expressed as the mean±standard deviation (SD). The method showed an intra-day and inter-day precision with coefficients of variation lower than 11% for all drugs. The lower limit of quantification (LOQ) was 5 ng/ml for all drugs.

### 3.3. Extraction efficiency

Over the concentrations studied, the minimum recoveries (mean±SD, $n = 3$) were 69.1±7.7, 33.5±5.6, 61.7±1.9, 92.7±5.3, 72.4±3.2 and 69.0±3.9% for OLZ, OH-RSP, RSP, I.S., CLZ and HLP, respectively.

### 3.4. Stability

To determine the influence of temperature on the stability of drugs, quality control samples spiked with five drugs (30–200–600 ng/ml, $n = 4$) were stored in different conditions: at −20 °C during 30 days; at +4 °C during 72 h; at +20 °C during 24 h and after three repeated freeze–thaw cycles.

Storage at room temperature for 24 h showed good stability of each compound, as evidenced by the percent coefficient of variation less than 16% and the accuracy bias of less than 10%. Storage at room temperature and light for 24 h produce significant degradation of each compound with accuracy bias of more than 20%. Five compounds appeared to be stable after three repeated freeze–thaw cycles with C.V. and % bias values less than 10%. No decomposition was noted in the frozen samples over 30 days. Storage for 72 h at +4 °C produced no significant decrease in concentration.

Plasma extracts were stable in mobile phase for at least 24 h at +4 °C in the HPLC unit.

### 3.5. Specificity

Potential interferences with determinations were investigated by assaying different blank plasma spiked with drugs commonly found in voluntary drug intoxications. No significant chromatographic interferences were found with tested tricyclic antidepressants (amitriptyline, imipramine), selective serotonin reuptake inhibitors (fluoxetine, paroxetine), benzodiazepines (diazepam, bromazepam, zopiclone, flunitrazepam) and neuroleptics (chlorpromazine, thioridazine).

### 3.6. Clinical cases

By using the method described, we were able to manage several cases of antipsychotic poisoning.

Typical patient chromatograms are shown in Fig. 2. In Fig. 2A, risperidone and its metabolite were identified by their ultraviolet absorption spectrum and then quantified. Risperidone and metabolite concentrations were 220 and 82 ng/ml, respectively, way above the therapeutic concentration (1–28 ng/ml) [20].

Fig. 2B shows a chromatogram from a patient in whom olanzapine was identified and quantified. Olanzapine concentration was 286 ng/ml (therapeutic concentration range is 20–40 ng/ml) [21].

In conclusion, this screening procedure is easy to perform and allows for rapid quantification and identification of atypical antipsychotics and haloperidol. The limit of quantification and short duration of the assay are particularly adapted to the management of antipsychotic overdoses.

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