patients with the data for 13 adult SARS patients who had been studied in a previous report (7). The adult plasma samples were taken at a mean of 4 days after fever onset (range, 2–6 days) and exactly 7 days after fever onset. The median adult plasma SARS-CoV RNA concentration at a mean of 4 days after fever onset was 125 copies/mL. We observed no significant difference between the plasma SARS-CoV RNA concentration in the pediatric patients and that in the adult SARS patients (Mann–Whitney test, $P = 0.076$). In addition, we compared the plasma SARS-CoV RNA concentration at day 7 after fever onset. The median adult plasma SARS-CoV RNA concentration at day 7 was 84 copies/mL. Once again, we observed no significant difference between pediatric and adult SARS patients (Mann–Whitney test, $P = 0.076$). Because the number of patients in this study was limited, further study involving more patients may be necessary to address the difference of viral loads between adult and pediatric SARS patients.

In this study we demonstrated that SARS-CoV RNA is detectable in the plasma of pediatric SARS patients with a detection rate of 87.5–100% within the first week after fever onset and then dropped to 62.5% at a mean of 14 days after fever onset. These data are largely concordant with our previous data on adult SARS patients showing a high detection rate for serum SARS-CoV RNA within the first week of illness (7). Taken together, these data suggest that plasma SARS-CoV measurement is a sensitive method for detecting SARS-CoV infection during the first week of fever onset.

The serial data presented here have demonstrated that SARS-CoV RNA in plasma from the studied patients became undetectable after a mean of 16 days of fever (range, 9–21 days). For patient 7, the undetectable plasma SARS-CoV RNA at days 4 and 10 might represent a fluctuation in the degree of viremia during the course of the illness as a result of intermittent shedding of virions. We did not observe any correlation between the plasma viral load and steroid or ribavirin treatment, and a larger scale study may be necessary to address this important question.

Recent studies have reported that the clinical course is less severe in pediatric SARS patients than in adult SARS patients (8, 9). A logical question would be whether the plasma SARS-CoV viral load in pediatric SARS patients is different from that in adult SARS patients. When we compared the data from pediatric patients with data from adult SARS patients (7), we observed no significant differences in plasma SARS-CoV viral load in samples taken from pediatric and adult SARS patients within the first week of admission and at day 7 after fever onset.

In conclusion, viremia appears to be a consistent feature in both pediatric and adult SARS patients. The relatively high detection of SARS-CoV in plasma during the first week of illness suggests that plasma-based RT-PCR may potentially be useful in the routine diagnostic work-up of patients with suspected or confirmed SARS in both adult and pediatric populations.

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References


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Applicability of an Assay for Routine Monitoring of Highly Variable Concentrations of Olanzapine Based on HPLC with Mass Spectrometric Detection, Guillermo Gerosaﬁni,1 Sonia Vizcaino,1 Angustias G. Herráiz,2 Julio Benitez,1 and Juan Antonio Carrillo1 (1 Department of Pharmacology and Psychiatry, Extremadura University School of Medicine, E-06071 Badajoz, Spain; 2 Psychiatric Hospital Adolfo Díaz Ambrona, E-06800 Mérida, Spain; * address correspondence to this author at: Departamento de Farmacología y Psiquiatría, Facultad de Medicina, Universidad de Extremadura, Avda. de Elvas s/n, E-06071 Badajoz, Spain; fax 34-924-271100, e-mail carrillo@unex.es)

The thienobenzodiazepine derivative olanzapine (OLZ) is a commonly used antipsychotic drug that has demonstrated efficacy against both positive and negative symptoms of schizophrenia (1).

OLZ shows variable pharmacokinetics, with induction of the CYP1A2 enzyme by cigarette smoking being one of the most important factors contributing to this variability (2). Moreover, because of the potential toxicity of OLZ at relatively low concentrations, monitoring of OLZ has been suggested to be necessary (3).
Several methods have been developed to measure OLZ in whole blood, plasma, or serum, including HPLC-based techniques (4–10), liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) (11–13), and gas chromatography coupled with MS (14).

The goal of this work was to develop a rapid and technically simple assay based on LC coupled with electrospray MS that would be capable of routinely determining extremely low OLZ concentrations. This could be very useful for monitoring schizophrenic patients who are heavy smokers, a group that accounts for 70–90% of these patients (15), who are more likely to display lower OLZ concentrations than nonsmokers (2, 16).

The chromatographic system used consisted of a Hewlett-Packard (HP) 1100 series equipment (Agilent Technologies Spain S.L.) with a degasser (Model G1322A), quaternary pump (Model G1311A), autosampler (Model G1313A), ultraviolet/visible detector (Model G1315A) set at 260 nm wavelength, and a quadrupole mass spectrometer (model G1946A). A computer-assisted HP G2710AA LC/MS ChemStation (Agilent Technologies Spain S.L.) was used to operate the modules and facilitate data management.

Blood samples were collected in tubes containing EDTA, and plasma was immediately separated after centrifugation and stored frozen at −20 °C until assayed. To avoid OLZ oxidation during storage and manipulation (6), we added 5 μL of a 250 g/L ascorbic acid solution to each sample (final concentration, 2.5 g/L).

OLZ was extracted from 0.5 mL of plasma, whereas many previously published OLZ extraction methods use 1 mL as starting sample volume (4–7, 9, 10, 14). The process was also time-saving, being ready in a single step as follows: 75 ng/75 μL of the internal standard (IS)
LY170222 and 500 μL of 0.5 mol/L NaOH were added to the plasma; the samples were slowly mixed and extracted with 2 mL of ethyl acetate for 10 min and then centrifuged. The organic phase was evaporated to dryness at 37 °C under a stream of nitrogen. After reconstitution of the residue in 75 μL of mobile phase, 30 μL was injected on a HyperSil BDS C₁₈ \( [125 \times 3.0 \text{ mm (i.d.); 3 μm particle size} \text{ reversed-phase column (Agilent Technologies Spain S.L.) and eluted isocratically with a mobile phase containing 780 mL/L formic acid (1 g/L) and 220 mL/L acetonitrile at a flow rate of 0.4 mL/min. OLZ was subsequently detected in the electrospray ionization-MS system, and the mass spectrometer was run in the positive ion mode. Nitrogen as the drying gas was supplied at a flow of 10 L/min at a temperature of 330 °C. The capillary voltage was adjusted to 4000 V. The HPLC eluant was introduced in the source via the electrospray interface, generating the positively charged pseudomolecular ion \([M+H]^+\). To establish the appropriate selected-ion monitoring (SIM) conditions, we collected full-scan mass spectra of OLZ and IS in the range of 50–400 atomic mass units (amu) at different fragmentor voltages (range, 55–180 V). On the basis of observed fragmentation, the fragmentor was set at 105 V for SIM detection of the drug and IS. The following ions were monitored: \(m/z\) 313 and 256 (protonated molecular peak and fragment of OLZ), and \(m/z\) 327 and 270 (protonated molecular peak and fragment of the IS; Fig. 1, A–C).

At the above-mentioned conditions, the retention times of OLZ and IS were 3.2 and 4 min, respectively (Fig. 1, B and C), thus reducing the time of analysis to <5 min. The length of the analysis is the same as, and often shorter than, the lengths of analysis for other reported assays (4–7, 10, 11, 13, 17).

For calibration, 10 calibration curves were prepared by adding a working stock solution of 2 μg/L OLZ (10 mg/100 mL of methanol) to drug-free plasma, to give concentrations of 0.1, 1, 5, 10, 50, 100, and 200 μg/L. OLZ plasma concentrations were quantified by linear regression [peak-area ratio of OLZ to the IS LY170222 \((m/z\) 327)]. The presence of fragment peaks was used for confirmation purposes.

To evaluate the precision and reproducibility of the assay, quality-control (QC) samples were prepared by adding the OLZ to drug-free plasma to final concentrations of 3, 40, and 125 μg/L. Calibrators and QC solutions were stored at −20 °C until assayed. The IS was prepared by dissolving 5 mg in 50 mL of methanol and then diluting with water to a concentration of 1 mg/L; the IS was stored at −20 °C.

Calibration curves were linear within the range of concentrations established (0.1–200 μg/L) with mean (SD) correlation coefficients \((r)\), \(y\)-intercepts, and slopes of 0.9964 (0.0029), 0.009 (0.0040) μg/L, and 0.213 (0.073), respectively. The reproducibility and precision of the assay were determined by analysis of the QC samples; these controls (3, 40, and 125 μg/L) were analyzed over 10 days with 10 different calibration curves and a protocol identical to that used for the assessment of patient samples. The mean interday CV was 8.7%. The mean intraday CV (determined by analyzing six samples of drug-enriched plasma on the same day using one calibration curve) for OLZ concentrations was 5.4% (Table 1).

To test the efficiency of the single-step extraction procedure, we calculated absolute recoveries by dividing the peak areas for extracted plasma samples by those obtained from injection of unextracted QC samples (3, 40, and 125 μg/L) in mobile phase. The mean (SD) recoveries, based on 10 different assays of the QC samples, were 94 (3.5)% for OLZ and 97 (4.2)% for the IS LY170222. The lowest limit of quantification was defined as the lowest concentration on the calibration curve with an acceptable accuracy of at least 100 (20)% \([(\text{mean assay concentration/ theoretical concentration}) \times 100\%]\) and relative standard deviation of no more than 20%. The lowest limit of quantification for OLZ obtained in this assay was 0.1 μg/L (Fig. 1C). This limit is lower than those reported for other HPLC or MS-based methods (4, 5, 7–11, 13, 18).

The detection limit of the assay, defined as the lowest quantity of an analyte that can be detected at a signal-to-noise ratio of 10, was 0.05 μg/L.

The specificity of the assay was demonstrated by the absence of endogenous substances, in drug-free plasma, that interfered with quantification of OLZ and IS (Fig. 1A). Furthermore, to test putative interferences from other possibly coextracted substances, we analyzed an array of psychotropic drugs that could be concomitantly administered along with OLZ, e.g., bromazepam, caffeine, carbamazepine, clonazepam, clorazepate, clozapine, diazepam, doxepin, fluoxetine, fluvoxamine, haloperidol, imipramine, levomepromazine, lorazepam, midazolam, nortriptyline, quetiapine, risperidone, and thioridazine. None of these drugs was found to interfere with the analysis of OLZ or the IS in terms of ion suppression or contribution to monitored ions.

Ion suppression effects have been claimed to affect the quantitative performance of MS methods (19). We therefore tested the potential deleterious effects of endogenous matrix components, using 0.5-mL aliquots of plasma subjected to four different extraction techniques. Results (matrix effect) were compared with the relative 100% signal response obtained from injection of mobile phase.

<table>
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<tr>
<th>Table 1. Results of intra- and interday precision study for OLZ.</th>
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<tbody>
<tr>
<td>OLZ</td>
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<tr>
<td>Overall</td>
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<tr>
<td>Intraday (n = 6)(^a)</td>
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<tr>
<td>Mean, μg/L</td>
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<tr>
<td>SD, μg/L</td>
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<td>CV, %</td>
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<tr>
<td>Interday (n = 10)(^a)</td>
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<tr>
<td>Mean, μg/L</td>
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<tr>
<td>SD, μg/L</td>
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\(^a\) Number of determinations.
with 50 μg/L OLZ added. As can be seen in Fig. 1D, extracts from protein precipitation and solid-phase methods showed a substantial reduction in instrument response compared with mobile phase. In contrast, the ethyl acetate liquid-liquid preparation used in our assay suppressed the signal of the analytes by <3%.

This assay, because of the high simplicity of the extraction procedure, the short duration of the analysis, the small amount of starting sample needed, and its high selectivity, is being used routinely by our research group to monitor plasma OLZ concentrations in schizophrenic patients. In fact, in a set of 17 smoking and nonsmoking patients, a wide drug concentration range was found [0.8–71 μg/L; mean (SD), 34 (4) μg/L for doses between 5 and 10 mg/24 h], consistent with the large variability of OLZ pharmacokinetics reported previously (16).

The high sensitivity shown by this assay makes it suitable for measuring the extremely low concentrations of OLZ in schizophrenic patients who are heavy smokers. It could also potentially be helpful in clarifying the clinically relevant role played by smoking, through increases in the clearance of OLZ via CYP1A2, and in demonstrating the marked variability of the antipsychotic response in schizophrenic patients (2). Likewise, the method could potentially be very useful for monitoring those patients who are given other drugs capable of inducing OLZ metabolism (e.g., anticonvulsants and barbiturates).

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Anti-Tissue Transglutaminase Antibodies in Arthritic Patients: A Disease-specific Finding? Antonio Picarelli;" Marco Di Tola," Luigi Sabbatella, Stefania Vetrono, Maria Cristina Anania, Antonio Spadaro," Maria Laura Sorgi," and Egisto Taccari (1 Gastroenterological Unit, Department of Clinical Sciences, and 2 Rheumatological Unit, Department of Medical Therapy; University “La Sapienza”, 151-00161 Rome, Italy; * address correspondence to this author at: Department of Clinical Sciences, Policlinico “ Umberto I”, University of Rome “La Sapienza”, Viale del Policlinico, 155-00161 Rome, Italy; fax 390649970524, e-mail a.picarelli@flashnet.it) Tissue transglutaminase (tTG), widely distributed in human organs, is a multifunctional enzyme involved in the cross-linking of extracellular matrix proteins, fibrogenesis, and wound healing (1). Recently, tTG has been proposed as the autoantigen of anti-endomysial antibodies (EMAs) (2), a serologic marker of celiac disease (CD) (3). Use of anti-tTG antibodies has been advocated in the diagnostic work-up of CD (4), although positive results have been reported in patients with other intestinal disorders, such as inflammatory bowel disease (5–7).

The aim of the present investigation was to evaluate the