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Short communication

# Quantitative determination of imidazenil, a novel imidazobenzodiazepine carboxamide derivative, by normal-phase high-performance liquid chromatography

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**Abstract**

Imidazenil, an imidazobenzodiazepine carboxamide derivative, is a novel anxiolytic and anti-convulsant agent recently characterized as a partial allosteric modulator of $GABA_A$ receptors. Owing to the pharmacological and pharmacokinetic importance of plasma-level determination, a HPLC method has been developed. Imidazenil was extracted from a plasma sample after a partition with diethyl ether, using alprazolam as internal standard. The analysis was performed by a normal-phase HPLC method with UV detection at 255 nm. The limit of quantitation was 6 ng corresponding to 30 ng/ml of plasma concentration. This procedure has been successfully applied to the quantitation of imidazenil plasma levels in primarily pharmacokinetic studies after a single i.v. and an oral administration of the compound to the rat.

*Keywords:* Imidazenil; Imidazobenzodiazepine carboxamide

## 1. Introduction

Imidazenil, 6-(2-bromophenyl)-8-fluoro-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxamide, (Fig. 1), is a novel compound acting as a partial agonist at benzodiazepine (BZD) receptors [1]. Imidazenil has shown neuropsychopharmacological effects in various animal models, predictive of anxiolytic and anti-convulsant activities [1,2].

Classic BZDs are known to produce anxiolytic, anti-convulsant, sedative, myorelaxant effects, to potentiate alcohol and to impair cognitive processes at overlapping dose ranges, while imidazenil appears to be predominantly anxiolytic and anti-convulsant

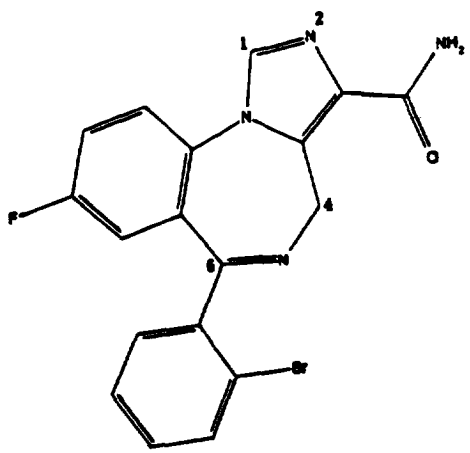


Fig. 1. Structural formula of imidazenil.

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[1]. Moreover, it has been reported to have a lower liability, if any, to induce tolerance and dependence [3,4]. Development of tolerance and dependence is indeed a major factor limiting the use of BDZs for long-term treatment of epilepsy, anxiety and insomnia.

Interestingly, imidazenil exhibits an anti-conflict activity comparable with that obtained with diazepam and bretazenil, a previously characterized BZD partial agonist [5], but a more potent anti-proconflict action [6]. This finding suggests a potential selective anti-panic action of imidazenil in humans [7].

To undertake preliminary pharmacokinetic studies of this new interesting compound in the rat, a HPLC method has been developed, with sufficient sensitivity to evaluate the plasma profile of imidazenil after a single i.v. and an oral dosing.

## 2. Experimental

### 2.1. Reagents and solvents

Diethyl ether and acetic acid were analytical grade; ethanol and *n*-hexane were HPLC grade. All solvents were purchased from Fluka (Buchs, Switzerland).

Imidazenil was obtained from Hoffmann-La Roche (Nutley, NJ, USA) while alprazolam was a gift from Upjohn Co. (Kalamazoo, MI, USA).

### 2.2. Sample preparation

Stock solutions of alprazolam as internal standard (i.s.) and imidazenil were prepared by carefully weighting about 10 mg of each compound into a 100-ml volumetric flask and dissolving with ethanol. The i.s. tested solution was obtained by diluting the stock solution at 1 $\mu$g/ml concentration, while serial dilutions of the imidazenil stock solution were used to make the tested standard solutions in the range between 50 ng/ml and 10 $\mu$g/ml. For all dilutions deionized water was used.

In centrifuge tubes, 200 $\mu$l of rat plasma, 100 $\mu$l of i.s. tested solution and 100 $\mu$l of imidazenil solutions at various concentrations were added. Diethyl ether (2 ml/sample) was added, the tubes were mixed for 15 min by a reciprocating shaker (Mischer 5432, Eppendorf, Fremont, CA, USA) and then centrifuged for 10 min at 2500 $\times$ *g*. The organic phase was transferred to another tube and evaporated to dryness under a stream of nitrogen. The residue, dissolved in 100 $\mu$l of mobile phase was injected into the HPLC apparatus.

### 2.3. Instrumentation

The HPLC system (Perkin-Elmer, Norwalk, CT, USA) consisted of a pump (LC 410), a UV detector (LC 235 Diode Array), an autosampler (ISS 100) and a data acquisition system (Nelson software). A Lichrosorb-CN column (250 $\times$ 4 mm I.D., 5 $\mu$m; Merck, Darmstadt, Germany) was used.

### 2.4. Chromatographic analysis

Chromatography was performed in normal-phase mode, using a mobile phase composed of *n*-hexane–ethanol–acetic acid (78:17.6:4.4, v/v/v) at a 1.5 ml/min flow-rate. The detector was operated at 255 nm.

Under such conditions the retention times for imidazenil and alprazolam (i.s.) were 7.8 and 12.5 min, respectively. No interferences of co-extracted plasma components were present in the region of chromatogram where imidazenil and i.s. eluted.

Standard calibration curves (ratio of imidazenil to i.s. peak areas versus imidazenil concentration) were obtained for samples into which known amounts of imidazenil and a constant quantity of i.s. were added, both in the presence or absence of the plasma matrix (i.e. after extraction or without extraction).

The recovery of imidazenil from plasma was calculated by comparing the two curves. The intra-day and the inter-day accuracy and the precision were calculated for replied samples at different imidazenil concentrations, including the quantitation limit.

## 3. Results and discussion

The analytical recovery (seven different concentrations were tested), calculated by comparing the

Table 1
Precision and accuracy of imidazenil recovery from plasma samples

| Expected concentration (ng/ml) | Observed concentration (ng/ml) | Number of replicates | Relative standard deviation (%) | Accuracy (%)[a] |
|---|---|---|---|---|
| *Intra-day* | | | | |
| 30.6 | 36.4 | 4 | 7.8 | +18.9 |
| 1224.0 | 1282.0 | 4 | 4.5 | +4.8 |
| *Inter-day (1 month)* | | | | |
| 1015.0 | 1118.0 | 6 | 12.2 | +10.1 |

[a]Differences between observed and expected concentration.

imidazenil/i.s. peak-area ratios of extracted plasma samples to the ratios obtained from control samples (i.e. no extraction), was quantitative and linear over the concentration range tested (30 ng/ml–12 μg/ml). The values of the slope and *y*-intercept were 0.00216 and −0.0168, respectively (correlation coefficient >0.9999).

Intra- and inter-day accuracy and precision data are reported in Table 1. It can be noted that the determination of an imidazenil plasma level close to the quantitation limit presents more uncertainty than the determination at a high plasma level, even if a linear curve (see above) including the response at low concentrations was obtained. For this reason, in order to minimize the uncertainty of the determination around the quantitation limit, imidazenil plasma concentrations in treated animals were calculated in comparison with a calibration curve (four points) for expected concentrated samples. The mean response of three replicates at the same low concentration (about 30 ng/ml) was used as a reference for the expected diluted samples.

In both cases, the samples were prepared using blank plasma of an untreated animal to which known quantities of imidazenil and i.s. were added before the extraction.

The proposed HPLC method was applied to determine imidazenil plasma concentrations in the rat

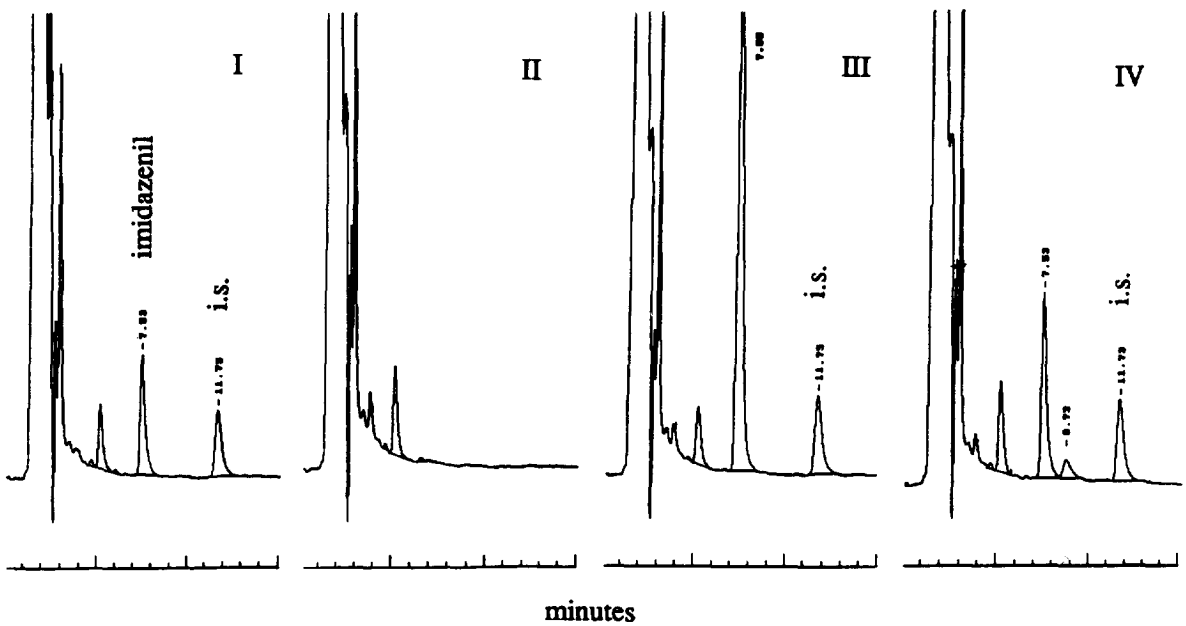


Fig. 2. Chromatograms of HPLC analysis of extracted plasma samples: authentic standards recovered from a control rat plasma (I), blank rat plasma (II), rat plasma following an i.v. dosing of imidazenil (4 mg/kg) after 5 min (III) and 3 h (IV) from the treatment.

following a single i.v. and oral dose of the compound during a preliminary pharmacokinetic study.

As an example we report two chromatograms obtained from a rat treated with 4 mg/kg of imidazenil by the i.v. route (Fig. 2). The original drug and another compound are present in the plasma. This compound is detectable after 15 min from the i.v. injection and peaks at 1 h. Some investigations are in progress to define the chemical structure of this probable metabolite, so that the specificity of the method can be fully evaluated.

The time courses of imidazenil plasma concentration in two treated rats are shown in Fig. 3.

Further applications of this technique were made for the quantitation of imidazenil in plasma from rats following i.v and oral administration at low doses (starting from 0.5 mg/kg) in an extended pharmacokinetic study in which the most important pharmacokinetic parameters and the systemic availability of imidazenil were calculated.

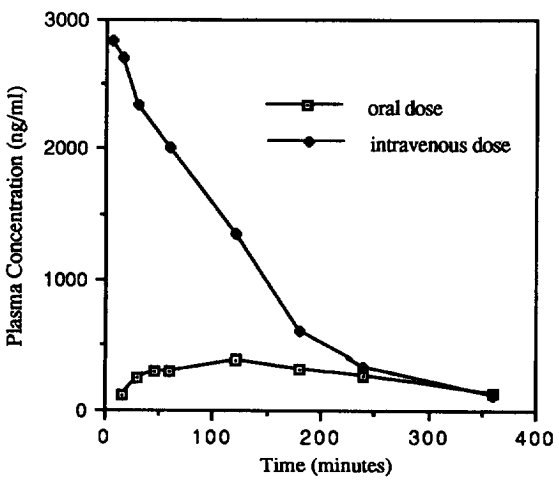


Fig. 3. Plasma levels time course of imidazenil in two rats after a single i.v. and an oral administration of the compound at a dose of 4 mg/kg.

The sensitivity of the method appeared satisfactory as the monitoring of plasma concentration was possible over three imidazenil half-lives, an appropriate period for a good description of a decay curve. An extensive pharmacokinetic treatment of the results will be the subject of a further paper.

At present, no pharmacokinetic studies have been made in man; however, a more sensitive analytical method should probably be set up, taking into account that very low doses of imidazenil seem to be adequate to produce therapeutic effects.

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## References

[1] P. Giusti, I. Ducic, G. Puia, R. Arban, A. Walser, A. Guidotti and E. Costa, J. Pharmacol. Exp. Ther., 266 (1993) 1062.
[2] M. Serra, C.A. Ghiani, C. Motzo, T. Cuccheddu, S. Floris, P. Giusti and G. Biggio, J. Pharmacol. Exp. Ther., 269 (1994) 32.
[3] J. Auta, P. Giusti, A. Guidotti and E. Costa, J. Pharmacol. Exp. Ther., 270 (1994) 1262.
[4] C.A. Ghiani, M. Serra, C. Motzo, P. Giusti, T. Cuccheddu, M.L. Porceddu and G. Biggio, Eur. J. Pharmacol., 254 (1994) 299.
[5] W. Haefely, Clin. Neuropharmacol., 7 (1984) 670.
[6] A. Guidotti, P. Giusti, R. Arban, G. Puia, I. Ducic and E. Costa, Soc. Neurosci. Abstr., 19 (1993) 523.5
[7] P. Giusti, G. Guidetti, E. Costa and A. Guidotti, J. Pharmacol. Exp. Ther., 257 (1991) 1062.