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Determination of several N-methyl-D-aspartate receptor blockers in plasma and brain by a selective high-performance liquid chromatographic method with column switching

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Abstract

A general procedure is presented for the determination of several N-methyl-D-aspartate (NMDA) receptor open-channel and subtype-selective blockers, which have been evaluated and developed as neuroprotective drugs for the treatment of brain stroke and trauma. The method involves deproteination of plasma with ethanol, or homogenization of brain samples in ethanol, dilution of the supernatant with ammonium acetate and direct injection into an HPLC column-switching system. Although the investigated NMDA receptor blockers are all tertiary amines, they have quite different structures. However, they are all concentrated on the first column (Purospher RP-18, 125×4 mm), whereas polar interfering compounds are washed out with 1% ammonium acetate–acetic acid–acetonitrile (100:1:5, v/v/v). Due to the special selectivity of the Purospher RP-18 material, the analytes and the internal standard are then selectively eluted with 25% acetonitrile (without any buffer in the mobile phase) and transferred to the analytical column (Superspher 60 RP-select B, 250×4 mm), where they are separated by gradient elution and detected by UV or fluorescence detection. The low degree of interference allowed the development of sensitive methods with quantification limits of 5 ng/ml for animal plasma (0.4 ml used), 0.5 ng/ml for human plasma (1 ml used) and 50 ng/g for brain tissue (200 mg used). © 1998 Elsevier Science B.V.

Keywords: Column switching; Methylaspartate receptor blockers

1. Introduction

Ro 25-6981 (**I**, Fig. 1) and Ro 24-6173 (**II**) are NMDA (N-methyl-D-aspartate) receptor subtype-selective [1] and open-channel blockers, respectively, which have been evaluated and developed as neuroprotective drugs for the treatment of brain stroke and trauma. To profile these drugs for the i.v. treatment of acute ischemic damage, analytical methods were needed for sample analysis from pharmacokinetic, neuroprotection, cardiovascular and neurotoxicity studies in animals, as well as a tolerance study in human volunteers.

In this report, a general procedure is presented for this class of compounds which consists of deproteination of plasma with ethanol, or homogenization of brain samples in ethanol, dilution of the supernatant with ammonium acetate and direct injection into an HPLC column-switching system. The procedure relies on a selective elution of the analytes from the first column (Purospher RP-18) with 25% acetonitrile without any buffer in the mobile phase. This was possible due to the extraordinary properties and selectivity of this stationary phase which was originally designed for the analysis of amines with

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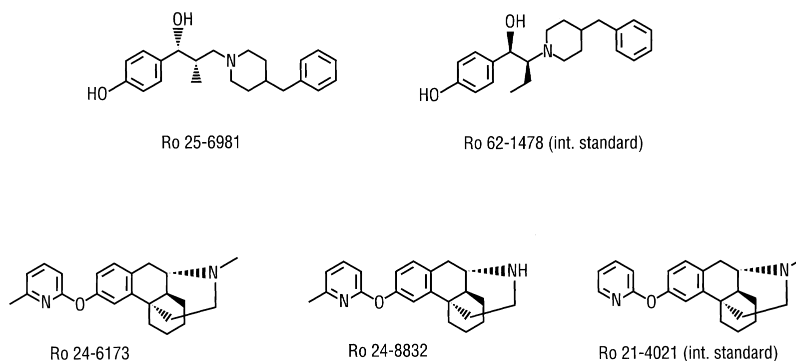

Fig. 1. Chemical structures of the compounds.

minimal peak tailing. Several examples of the determination of both drug candidates in different matrices and species are shown.

2. Experimental

2.1. Materials, reagents and solvents

Glacial acetic acid (100%) and ammonium acetate (both analytical-reagent grade), and ethanol (absolute analytical-reagent grade), were obtained from E. Merck (Darmstadt, Germany), and acetonitrile (HPLC grade S) from Rathburn (Walkerburn, UK). Water was distilled twice from an all-glass apparatus. Compounds **I** (Ro 25-6981/601), **IV** (Ro 62-1478/000, I.S.), **II** (Ro 24-6173/602), **III** (Ro 24-8832/001) and **V** (Ro 21-4021/003, I.S.) were provided by F. Hoffmann-La Roche (Basel, Switzerland). Spiked plasma samples were prepared using fresh frozen plasma, either from sodium citrated human blood, which was obtained from a blood bank (Blutspendezentrum SRK, Basel, Switzerland), or from EDTA/NaF blood from our own laboratories (rat, cat, dog and rabbit), from the Wellcome Surgical Institute and Hugh Fraser Neuroscience Laboratories, University of Glasgow (cat) or from Corning Hazleton (Münster, Germany; Cynomolgus monkey). Blank rat and dog brain was obtained from the Toxicology Department, F. Hoffmann-La Roche.

2.2. Solutions and standards

A 10% (w/v) solution of ammonium acetate was made up in bidistilled water (100 g/l). Stock solutions of the internal standards were prepared by dissolving 10 mg in 100 ml of ethanol (100 μg/ml). Internal standard working solutions were prepared by diluting 1 or 2 ml of the stock solutions with ethanol to 2000 ml (50 ng/ml for **V** and 100 ng/ml for **IV**). These solutions could be stored at room temperature for several weeks. The stock solutions could be stored at 4°C for several months.

A stock solution of **I** for plasma determinations was prepared by dissolving 13.42 mg (corresponding to 10 mg of free base) in 100 ml of ethanol. Stock solutions of **II** and **III** were prepared by dissolving 22.1 mg and 26.94 mg, respectively, (corresponding to 20 mg of free base) in 100 ml of ethanol. Ultrasonication was used for complete dissolution. The stock solutions were diluted with ethanol to give working solutions in the range 100–0.5 μg/ml. These working solutions were used as calibration standards for the animal plasma assays by adding 0.5 ml to 49.5 ml of human plasma, yielding concentrations of 1000, 500, 200, 50, 10 and 5 ng/ml. For the human plasma assay for **II**, working solutions in the range 20–0.05 μg/ml were prepared and diluted accordingly, yielding plasma calibration standards of 200, 100, 20, 4, 0.8 and 0.5 ng/ml. All plasma calibration standards could be stored at −20°C for several months. Spiked plasma standards for validation or quality control samples were prepared by spiking blank plasma with a small volume (normally 1%) of an adequately prepared standard working solution.

A stock solution of **I** for brain determinations was prepared by dissolving 6.71 mg (corresponding to 5

mg of free base) in 100 ml of ethanol. Ultrasonication was used for complete dissolution. The stock solution was further diluted with ethanol. A 10-μl volume of the stock solution or of the dilutions were added to 200 mg of homogenized blank dog brain, resulting in concentrations of 2500, 1000, 500, 250, 100 and 50 ng/g. The brain calibration standards were prepared freshly before use. Spiked brain homogenate samples for validation or use as quality control samples were prepared in a similar way.

2.3. Sample preparation

To 0.4 ml of animal plasma, 1.2 ml (for **I**) or 1.5 ml (for **II** and **III**) of the internal standard working solution were added for protein precipitation. After vortex-mixing and storing for 15 min in the deep freezer at −20°C, the sample was centrifuged (6 min at ca. 18 000 *g* and 10°C), and the supernatant transferred to a 4-ml autosampler vial containg 2 ml of 2% ammonium acetate–ethanol (100:2, v/v). After mixing, 3.3 ml or 3.5 ml were injected.

For the human plasma assay of **II** and **III**, 1.0 ml of human plasma was deproteinated with 2.0 ml of ethanol (containing the I.S. **V**), treated as above, and 4.2 ml were injected.

To brain tissue (ca. 200 mg), 0.2 ml water and 1.5 ml ethanol were added, followed by homogenization using a Polytron (Model PT 1200C; Kinematica, Littau–Luzern, Switzerland; aggregate PT-DA 1207/2, 7 mm). After adding the internal standard **IV** and storing for at least 15 min in the deep freezer at −20°C and then centrifugation, the supernatant was added to 2.0 ml of 2% ammonium acetate–ethanol (100:2, v/v). After mixing, 3.5 ml were injected.

2.4. Chromatographic system and conditions

A schematic representation of the HPLC column-switching system is given in Fig. 2. An HPLC pump L-6000 (P1A; Merck), in combination with a solvent selector (SS; Labsource, Reinach BL, Switzerland), delivered mobile phase M1A (or alternatively M3). Aliquots (3.3 to 4.2 ml) were injected by the autosampler (AS; Model AS-4000A, Merck) onto the precolumn (PC). In order to inject large sample volumes, the autosampler was used with two 5-ml syringes as dilutors 1 and 2 (solvents: water–ethanol (100:4, v/v) and ethanol, respectively), a 6-ml sample loop, and the slow needle-down-speed. The UV detector D1 (Spectroflow 773, Kratos, Ramsey, NJ, USA), operating at 230 nm, together with a W+W recorder 600 (Kontron, Zurich, Switzerland), was used during method validation to monitor the removal of polar components from the precolumn during the purge step; they were not needed for routine analysis. The pre-concentrated analytes and internal standard were eluted with mobile phase M1B using HPLC-pump P1B (L-6000, Merck; flow-rate 1.0 ml/min) and a T-piece (Valco Instruments, Houston, TX, USA; 1/16 in., bore 0.25 mm). The gradient pump P2 (L-6200A, Merck) delivered mobile phase M2, which was degassed on-line (Solvent degaser SDU 2003, Labsource). A manual injector (MI; Model 7125 with a 200-μl loop, Rheodyne, Cotati, CA, USA) was used for direct injection onto the analytical column (e.g. for recovery experiments). Detection of the eluted compounds was carried out at 275 or 280 nm with an UV detector (D2; SPD-10A, Shimadzu, Kyoto, Japan; response value 1.0 s, aux range 3 AU/V). Alternatively, a Waters 474 Scanning Fluorescence Detector (Waters, Milford, MA, USA; excitation 276 nm, emission 306 nm, gain 10, DC filter 5 s) was used.

An HPLC ChemStation (Hewlett–Packard, Waldbronn, Germany), in connection with a laboratory-developed LIMS (UNICHROM and KINLIMS [2]), was used for data acquisition and processing. The two electrically-driven switching valves (V1 and V2; High speed valve 7000E, both Labsource) and the solvent selector were controlled by P2.

2.5. Columns and mobile phases

LiChroCART HPLC cartridges (all 4×4 mm I.D.) packed with LiChrospher 100 RP-18 (5 μm) were used as guard columns, GC1A and GC1B, and a LiChroCART HPLC cartridge (125×4 mm I.D.) packed with Purospher RP-18 (5 μm) was used as precolumn (PC). The analytical column (AC) consisted of a LiChroCART HPLC cartridge (250×4 mm I.D.) packed with Superspher 60 RP-select B (4 μm) (all Merck) and was kept at 25°C by a column thermostat (CT; with Peltier controller unit PCU 1000, Labsource).

Mobile phase 1A (M1A) was prepared by mixing

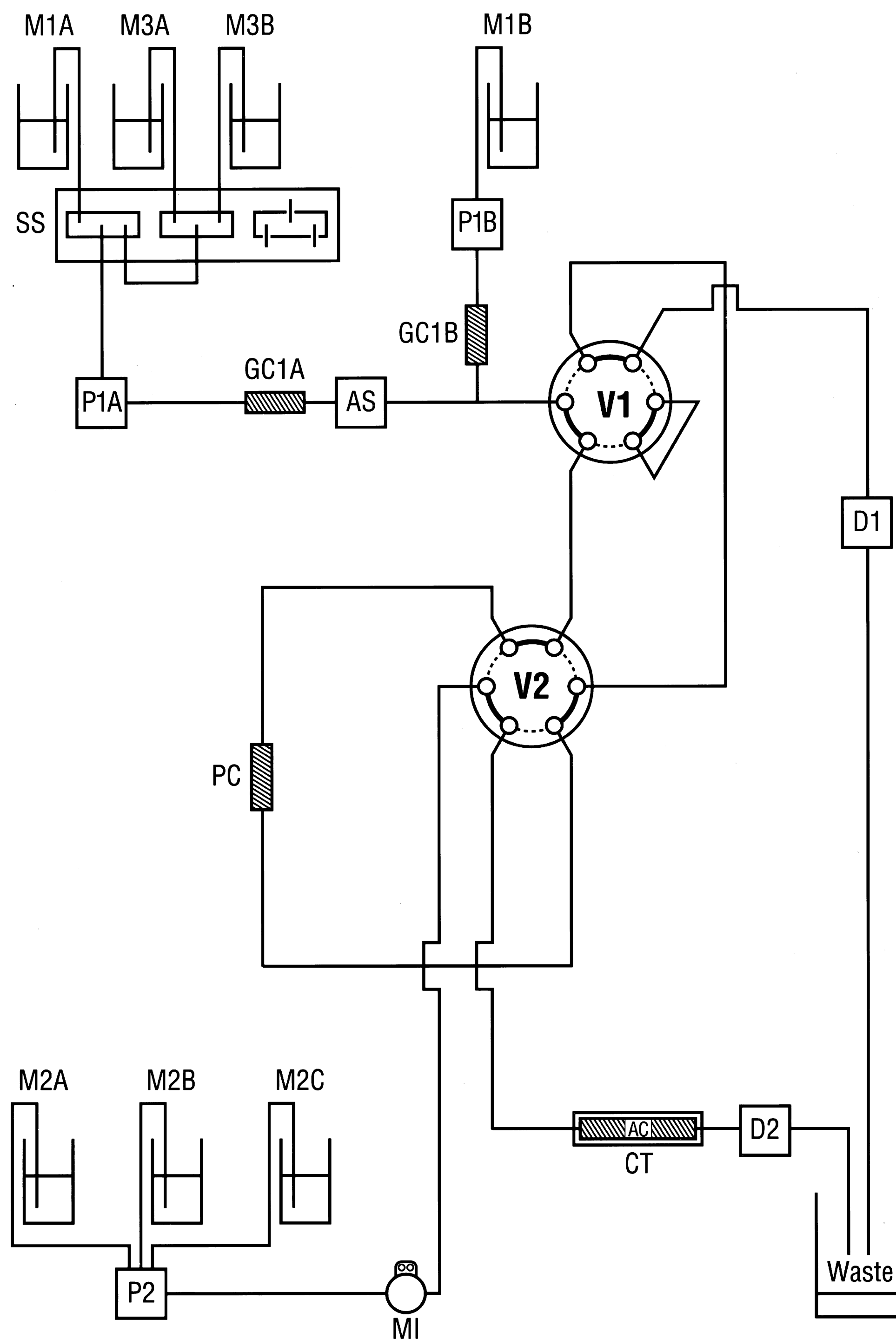

Fig. 2. Schematic representation of the HPLC column-switching system. See Section 2.4 for further details.

100 ml of 10% ammonium acetate with 900 ml of water, 50 ml of acetonitrile and 10 ml of acetic acid. Mobile phase 1B (M1B) was prepared by mixing 250 ml of acetonitrile with 750 ml of water. Mobile phase 2 (M2) consisted of three components (see chromatograms for details). Mobile phase 3A (M3A) and 3B (M3B) consisted of acetonitrile–water (9:1, v/v) and ethanol, respectively.

2.6. Procedure

The total sequence of automated sample analysis required 32 min. Details of the parameters used are shown in Table 1. The autosampler started pump 1B, the gradient program of P2 and the HPLC Chem-Station. P2 controlled the flow of P1A. In addition, the timer signals of P2 were also used for: (a) switching the valves (10/11 and 20/21 means switching of V1 and V2, respectively), (b) the solvent selector (30=M1A, 31=M3 (either M3A or M3B), 40=M3A, 41=M3B), and (c) to start (52) or to stop (62) P1B.

2.7. Calibration and calculations

Together with the unknown and quality control samples, six calibration standards, distributed over the whole set of samples, were processed as described above. The calibration curve ($y=a+bx$) was obtained by weighted linear least-squares regression (weighting factor $1/x^2$) of the measured peak-height ratios analyte/I.S. (y) versus the concentration of the analyte (x).

3. Results and discussion

3.1. Sample preparation

An automated HPLC column-switching system,

Table 1
Column-switching parameters and gradient program

Time (min)	P2 gradient			P2 flow (ml/min)	P1A flow (ml/min)	P2 timer				Comment
	A (%)	B (%)	C (%)			V1	V2	SS	Others	
0	100	0	0	0.05	0.05	10[a]	20			Injection of the sample onto PC using
0.1				1.0	1.0			30,40		M1A for pre-concentration of the
6.0				1.0	1.0					analyte.
6.1				0.2	2.0					
13.9				0.2	2.0					
14.0				0.7	0				52	PC is eluted with M1B.
15.2							21			Transfer of the retained components
15.5					2.0	11		31		from PC to AC. In the meantime, the
16.6							20		62	capillaries between AS and D1 are
16.7				1.0						purged with M3A...
17.0	100	0	0							
18.0								41		... and M3B..
18.9					2.0					
19.0					1.5					
21.9					1.5					
22.0					1.2			40		and again M3A.
24.0					1.0			30		Re-equilibration of PC with M1
26.0					1.5					
28.0	0	100	0							
30.0	0	100	0							
30.1	0	0	100		2.0					
31.8	0	0	100							
31.9	100	0	0			10				
32.0	100	0	0	1.0	2.0					

[a] The first digit of the timer signal represents the address and the second one the activation.

which had been used successfully for the determination of retinoids in biological samples [3,4], was adapted to the determination of NMDA receptor blockers. The only off-line step was deproteination of the biological fluids, using 2–4 volumes of ethanol. A large volume of ethanol results in more efficient protein precipitation, but also dilutes the sample. To decrease the elution strength of the supernatant, 2 ml of 2% ammonium acetate–ethanol (100:2, v/v) were added, prior to injection. The injection volume for animal plasma was 3.3 ml for **I** and 3.5 ml for **II** and **III**. For the human plasma method for **II** and **III**, 4.2 ml were injected to obtain maximum sensitivity. The low volume of ethanol in the dilution solution was used for stability reasons, as it prevents bacterial growth in this solution.

Cerebrospinal fluid and urine could be treated as plasma to avoid a change of the polarity of the injection solution, although these fluids contain only minimal amounts of proteins. To brain tissue (200 mg), 0.2 ml of water and 1.5 ml of ethanol were added, followed by homogenization and addition of the internal standard. Afterwards, the same procedure as for plasma was used.

3.2. Chromatographic system

The main difference in comparison with the retinoid column-switching system [3,4] was the use of a long precolumn (125×4 mm) packed with Purospher RP-18, instead of the 4 to 14 mm long precolumns with normal C_{18} material. The Purospher RP-18 precolumn allowed preconcentration with 1% ammonium acetate–acetic acid–acetonitrile (100:1:5, v/v/v), followed by a selective elution of the analytes and the internal standard with 25% acetonitrile. This resulted in a separation from plasma interferences when a precolumn with a certain length was used. A 4-mm Purospher RP-18 precolumn did not work for plasma samples. However, a 70-mm precolumn was sufficiently long to separate the analytes from interferences, allowing a reduction of the analysis time. Preliminary investigations using a 70-mm precolumn were successful [5]. However, at present, the 70-mm precolumn is only available on special request. About 500 injections of deproteinated plasma samples could be made onto one precolumn of 125 mm length before it was replaced. The preconcentration and selective elution also worked for cerebrospinal fluid and urine, as well as for brain samples.

3.3. Selectivity

As mentioned above, the use of the Purospher RP-18 precolumn allowed a selective separation of the analytes and the internal standard from plasma interferences. The procedure worked not only for **I** and other phenolic NMDA receptor blockers with similar structures, but also for **II** and **III** which have a morphinan structure. More experience is needed for a better understanding of the special selectivity of this precolumn material. However, all investigated compounds were amines. The Purospher RP-18 column was especially developed for the elution of amines with minimal interaction with metal ions and silanol groups [6]. Carboxylic acids appear to respond quite differently to the hydrophilic endcapping of this stationary phase [7].

In dog plasma, HPLC with UV detection showed a small interfering peak which was not present in other species. Fluorescence detection did not show any interference in dog plasma, and may be recommended for all species if such a detector is available.

Typical chromatograms for plasma and brain samples are shown in Figs. 3–6

3.4. Recovery

The recovery from plasma was determined during replicate analysis, by comparison of peak heights of spiked plasma samples, processed as described above, with aqueous standard solutions which contained the same amount of analyte and which were treated as the spiked plasma samples. The 100% values for **I** and **IV** obtained by injection onto the precolumn were also compared with solutions of the same amount of analyte directly injected onto the analytical column. For this study, the analyte and the internal standard were dissolved in mobile phase 2A, and 100 μl were injected ($n=8$). The peak heights were similar (94.2% and 104.2% for **I** and **IV**, respectively) to those in the experiment with precolumn injection, when the latter are defined as 100%.

The recoveries of compounds **I**–**III** were very

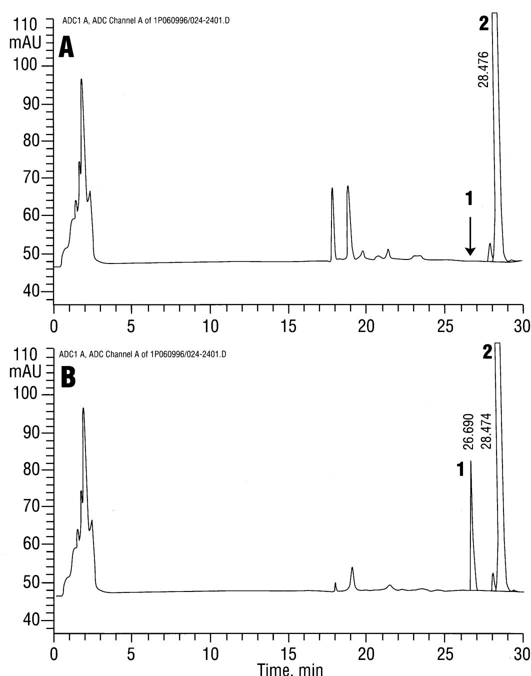

Fig. 3. Chromatograms of **I** in dog plasma: (A) Dog blank plasma containing the internal standard **IV** (peak 2). (B) Dog plasma sample taken 20 min after the start of an i.v. infusion of 14 mg/kg/2 h. Measured concentration of **I** (1) 645 ng/ml. M2A: 10% ammonium acetate–water–acetic acid–acetonitrile (50:680:20:250, v/v), M2B: ditto (50:480:10:500, v/v), M2C: ditto (5:20:5:980, v/v), D2: fluorescence detection, excitation 276 nm, emission 306 nm. Other conditions see Section 2.

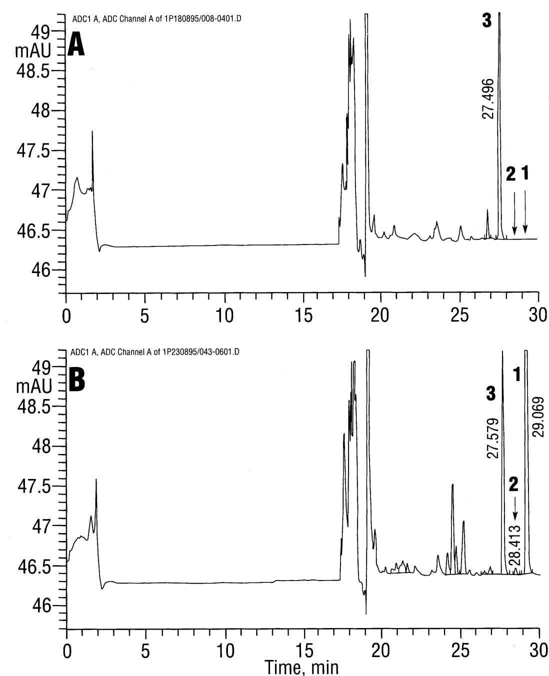

Fig. 4. Chromatograms of **II** and its N-demethyl metabolite **III** in rat plasma: (A) Rat blank plasma containing the internal standard **V** (3). (B) Rat plasma sample taken after 28 days of intermittent i.v. infusion of **II** (loading infusion of 7.78 mg/kg/h during 0.33 h followed by maintenance infusion of 2.16 mg/kg/h during 9.67 h daily for 4 weeks). Measured concentrations of **II** (1) 384 ng/ml and of **III** (2) 5.03 ng/ml. M2A: 10% ammonium acetate–water–acetic acid–acetonitrile (30:700:20:250, v/v), M2B: ditto (20:500:10:500, v/v), M2C: ditto (5:20:5:980, v/v), D2: UV detection, 280 nm. Other conditions see Section 2.

similar, independent of the plasma species. They ranged from 83.8% to 91.1% (Table 2).

The recovery from brain was determined during replicate analysis, by comparison of peak heights of spiked brain homogenates, processed as described above, with blank brain samples to which **I** and **IV** had been added prior to injection. The mean recovery for rat brain was 72.5% (Table 2). For dog and Cynomolgus monkey brain, similar recoveries were obtained (data not shown) but no detailed investigation was performed.

3.5. Linearity

The plasma methods, using either UV or fluorescence detection, were linear in the range 5–1000 ng/ml for animal plasma, and 0.5–1000 ng/ml for the human plasma conditions for **II** and **III**, at least. The brain method, using UV detection, was linear in the range 50–2500 ng/g, at least. Standard curves for the analytes in both matrices were calculated by means of weighted least-squares regression, using $1/x^2$ as weighting factor.

3.6. Limit of quantification

The limit of quantification for **I** in animal plasma samples was 5 ng/ml using 0.4 ml of plasma. The inter-assay (n=5) relative standard deviations (R.S.D.) at this concentration were 7.7% and 6.7% for rat and human plasma, respectively. The limits of quantification for **II** and **III** in animal plasma samples were 5 ng/ml using 0.4 ml of plasma. The inter-assay (n=4–5) R.S.D. were 1.1%–1.6% and

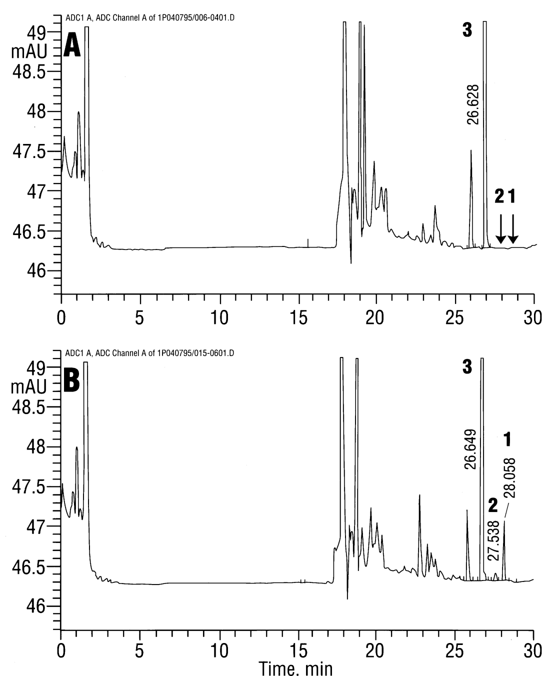

Fig. 5. Chromatograms of **II** and its N-demethyl metabolite **III** in human plasma: (A) Human blank plasma containing the internal standard **V** (3). (B) Human plasma sample taken 4 h after the end of a 6 mg i.v. infusion of **II** during 30 min. Measured concentrations of **II** (1) 9.51 ng/ml and of **III** (2) 1.15 ng/ml. M2A: 10% ammonium acetate–water–acetic acid–acetonitrile (30:700:20:250, v/v), M2B: ditto (30:500:10:500, v/v), M2C: ditto (5:20:5:980, v/v), D2: UV detection, 280 nm. Other conditions see Section 2.

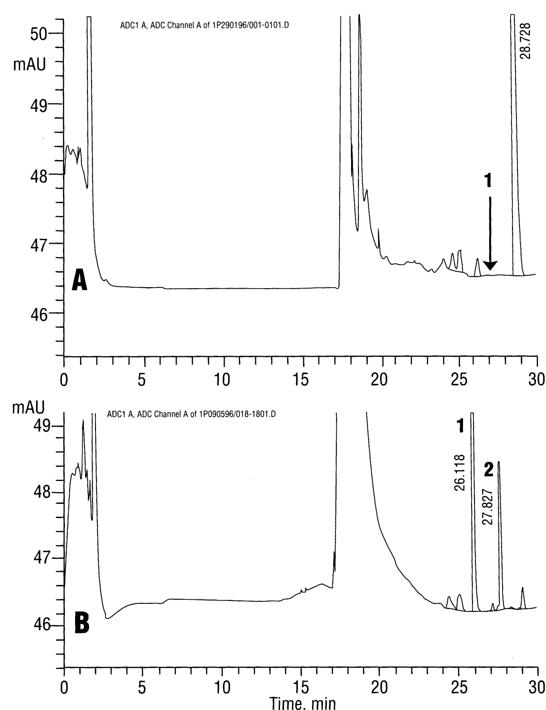

Fig. 6. Chromatograms of **I** in animal brain: (A) Dog blank brain. (B) Rat brain sample taken 5 min after the end of a bolus injection of 1.875 mg/kg within 2 min. Measured concentration of **I** (1) 3240 ng/ml. Peak 2 is the internal standard **IV**. M2A: 10% ammonium acetate–water–acetic acid–acetonitrile (50:680:20:250, v/v), M2B: ditto (50:480:10:500, v/v), M2C: ditto (5:20:5:980, v/v), D2: UV detection, 275 nm. Other conditions see Section 2.

0.9%–3.6%, respectively. For the human plasma method, the limit of quantification for **II** and **III** was 0.5 ng/ml. R.S.D. were 6.9% and 8.2% for **II** and **III**, respectively. Accuracies of the plasma methods were 90%–102% and 108% for animal and human plasma, respectively.

The limit of quantification for **I** in brain samples was 50 ng/g using 200 mg of brain. The inter-assay ($n=13$) precision and accuracy in dog brain samples spiked at this concentration were 10.8% and 97.8%, respectively.

3.7. Precision and accuracy

The inter-assay precision (defined as the relative standard deviation (R.S.D.) of replicate analyses) and the accuracy (defined as the deviation between found and added concentration) of the methods were evaluated over the calibration range in animal and human plasma, by analysing spiked samples on several days, using a separate calibration set on each day. The results are compiled in Table 3. The mean inter-assay precision for each method was 3.0% (**I**), 1.6% (**II**, animal plasma method), 1.4% (**III**, animal plasma method), 4.3% (**II**, human plasma method) and 3.3% (**III**, human plasma method).

The inter-assay precision in brain obtained from quality control samples was 3.0% and 3.8% and the accuracy 102% and 104% for rat and Cynomolgus monkey, respectively (Table 3).

3.8. Stability

Although only a limited stability investigation was

Table 2
Mean recoveries ($n=5$)

Species	Animal plasma method			Human plasma method		Brain tissue method
	I	**II**	**III**	**II**	**III**	**I**
Man	85.7%	88.5%	86.6%	85.8%	84.3%	
Rat	84.0%	85.8%	84.0%			72.5%
Cat	83.8%	85.5%	83.8%			
Monkey	88.4%	91.1%	89.6%			
Dog	84.9%	86.9%	86.0%			
Rabbit		88.1%	88.6%			

performed, all NMDA blockers proved to be stable at room temperature or −20°C in plasma from all investigated species. After storage at room temperature for 24 h, compound **I** showed a deviation from the added concentration of 6.1%, −2.7%, 5.4% and 0.3% for human, rat, cat and Cynomolgus monkey plasma, respectively. The deviations for compound **II** were 1.9%, −3.8%, 1.1% and −3.8%, and for compound **III** 1.2%, −3.2%, 0.7% and −3.2% for human, rat, Cynomolgus monkey and dog plasma, respectively.

When stored at −20°C for 3 months, the decrease in the concentration of compound **II** was −6.3%, −4.8% and −4.5%, and of compound **III** −6.7%, −5.2% and −4.9% for human, rat and dog plasma, respectively. As the 10% confidence intervals did not exceed −10%, the compounds were considered to be stable for these storage conditions [8].

3.9. Application of the method to biological samples

The methods described were successfully applied to the analysis of more than one thousand plasma and 50 brain samples from pharmacokinetic and toxicokinetic studies in rats, cats, dogs, Cynomolgus monkeys and rabbits, as well as in a tolerance study in volunteers. Figs. 4–6 show typical chromatograms from these studies, demonstrating the robustness of the assay.

4. Conclusions

A general procedure was found for the determination of several NMDA receptor open-channel and subtype-selective blockers consisting of deproteina-

Table 3
Inter-assay precision and accuracy

Species	Animal plasma method			Human plasma method		Brain tissue method
	I	**II**	**III**	**II**	**III**	**I**
Mean inter-assay precision ($n \geq 4$)						
Man	3.6%	0.7%	0.6%	4.3%	3.3%	
Rat	4.7%	0.8%	0.7%			3.0%
Cat	2.2%	3.6%	3.1%			
Monkey	3.0%	2.5%	2.1%			3.8%
Dog	1.7%	0.6%	0.6%			
Rabbit		1.3%	1.3%			
Mean inter-assay accuracy ($n \geq 4$)						
Man	98%	101%	100%	102%	102%	
Rat	99%	98%	99%			102%
Cat	97%	97%	98%			
Monkey	102%	101%	101%			104%
Dog	98%	99%	99%			
Rabbit		99%	100%			

tion of plasma with ethanol, or homogenization of brain samples in ethanol, dilution of the supernatant with ammonium acetate and direct injection into an HPLC column-switching system. The analytes were concentrated on a Purospher RP-18 column (125×4 mm), used as precolumn, whereas polar interfering compounds were washed out. Afterwards, the analytes and the internal standard were selectively eluted with 25% acetonitrile. Most of the plasma interferences which had been preconcentrated before were not eluted under these conditions. The low degree of interference allowed the development of sensitive methods with quantification limits of 5 ng/ml for **I** or **II** and its *N*-demethyl metabolite **III** in animal plasma (0.4 ml used), 0.5 ng/ml for **II** and **III** in human plasma (1 ml used) and 50 ng/g for all compounds in brain tissue (200 mg used).

UV detection was used for most determinations of compounds **I–III**, except for dog plasma for **I**, for which fluorescence detection was used because of better selectivity. However, fluorescence detection can also be used for the other species and is recommended for **I** and compounds with a similar phenolic structure. The procedure is also suitable for brain homogenates and cerebrospinal fluid or urine, although no validation was performed for these two bodyfluids. The methods for plasma and brain were applied to more than one thousand plasma and 50 brain samples from pharmacokinetic and toxicokinetic studies in rats, cats, dogs, Cynomolgus monkeys and rabbits, as well as in a tolerance study in volunteers. The procedure appears to be suitable for the determination of other NMDA receptor blockers and possibly amine drugs in general.

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