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Gas chromatographic–mass spectrometric assay for rocuronium with potential for quantifying its metabolite, 17-desacetylrocuronium, in human plasma

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Abstract

A rapid, sensitive and selective method has been developed for the quantification of plasma concentrations of neuromuscular blocking drug, rocuronium, using gas chromatography with mass spectrometric detection. 3-Desacetylvecuronium served as the internal standard. The method involved iodide ion pair formation and a single-step liquid–liquid extraction with dicholoromethane. This method also permits simultaneous determination of its putative metabolite, 17-desacetylrocuronium, although the high detection limit for the metabolite limits the practical application of this method in pharmacokinetic study of the metabolite. The extraction efficiency was ~75% for rocuronium and ~50% for 17-desacetylrocuronium. The limit of quantification was 26 ng/ml for rocuronium and 870 ng/ml for its metabolite. The assay was used successfully in a patient undergoing liver transplantation and receiving rocuronium as a constant rate infusion and in a patient undergoing general elective surgery receiving the drug as an intravenous bolus. This assay is a time-saving alternative to published gas or liquid chromatographic methods for assaying rocuronium.

Keywords: Rocuronium; 17-Desacetylrocuronium

1. Introduction

Rocuronium is a new quaternary aminosteriodal neuromuscular blocker (Fig. 1) which has rapid onset and short duration of action [1]. Relatively little information is available on its pharmacokinetics and pharmacodynamics due mainly to lack of suitable assays. It is postulated to have at least three metabolites, the major one being 17-desacetylrocuronium (Fig. 1). One published high-performance liquid chromatography (HPLC) method requires post-column extraction using a second pump device [2] and another published assay for rocuronium [3] modified a capillary gas chromatographic assay for steroidal blockers, vecuronium and pancuronium [4]. However, derivatisation of rocuronium and its putative metabolites was required

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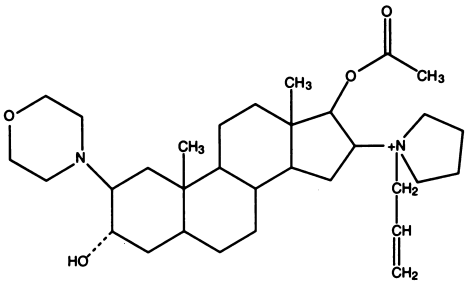
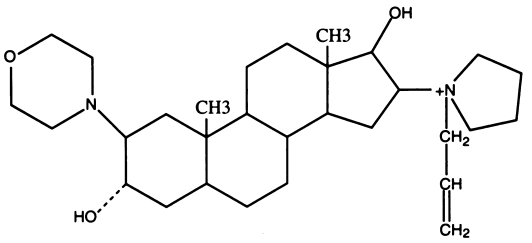
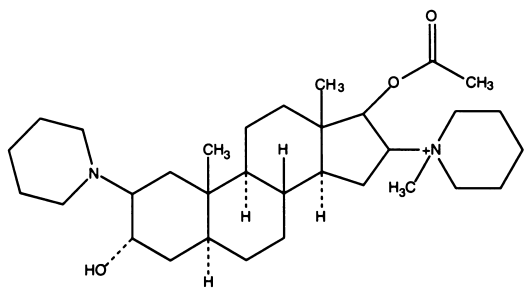


Rocuronium (Org 9426)



17-Desacetylrocuronium (Org 9943)



3-Desacetylvecuronium (Org 7268, IS)

Fig. 1. Chemical structures of rocuronium (Org 9426), 17-desacetylrocuronium (Org 9943) and the internal standard (I.S.) 3-desacetylvecuronium (Org 7268).

before sample injection and nitrogen sensitive detection, increasing the work-load during routine clinical analysis. A gas chromatographic–mass spectrometric (GC–MS) assay is reported here for the quantitative determination of rocuronium and its major putative metabolite, 17-desacetylrocuronium, in plasma. This method does not require derivatisation of the drug or its metabolite.

2. Experimental

2.1. Chemicals

Rocuronium bromide (Org 9426), 17-desacetylrocuronium bromide (Org 9943) and 3-desacetylvecuronium (Org 7268, internal standard, I.S.) were supplied by Organon Teknika (Boxtel, The Netherlands). Ethanol was purchased from Ajax (Sydney, Australia). Dichloromethane, sodium dihydrogen orthophosphate monohydrate, potassium iodide and 2-propanol were all analytical grade and obtained from BDH (Melbourne, Australia). Water was purified using an Alpha-Q filtration system (Millipore, Sydney, Australia).

2.2. Instrumentation

The gas chromatograph (GC-17A, Shimadzu Oceania, Sydney, Australia) was equipped with an autosampler (AOC 1400, Shimadzu) and a split/splitless advanced head pressure autoinjection system (AOC-17, Shimadzu). A DB-5 chemical bonded silica capillary column (15 m×0.25 mm I.D., film 0.25 μm; J&W Scientific, Folsom, CA, USA) was used. The column oven program was 120°C for 5 min, followed by heating at 30°C/min to 300°C where it was held for 10 min. The injector temperature was 280°C and a splitless injection mode was used; the split valve was opened after 30 s. The carrier gas was helium, linear velocity 46 cm/s. The column head pressure program started at 26 kPa for 5 min, followed by an increase at 6 kPa/min to 69 kPa where it was held to maintain a constant column flow-rate of 1 ml/min during the run. An electron impact (EI) mass spectrometer (QP-5000, Shimadzu) was used as a GC detector and operated under the following conditions: ionisation energy 70 eV, source temperature 280°C, scan speed 2000 amu/s and mass range, 40–600 Dalton. Peak area and height were integrated by LC-5000 data processing system.

2.3. Quantification of rocuronium

Relative GC retention time and EI mass spectra obtained by scanning within a 200 to 600 u range were used for identifying the compounds. Base peaks obtained from scanning were used for the quantification of rocuronium and 17-desacetylrocuronium by the selected ion monitoring (SIM) technique.

2.4. Stock and standard solutions

Intravenous (i.v.) injection solution of rocuronium bromide (10 mg/ml, equivalent to 8.69 mg/ml of rocuronium quaternary base) was used as the stock solution for rocuronium. Working standard solutions used for spiking drug free plasma were made fresh daily by diluting the stock solution with ethanol–water (20:1). This mixture (instead of water) had a pH of 5.5 and was more suitable for rocuronium stability. Authentic 17-desacetylrocuronium bromide (1 mg equivalent to 0.8592 mg of quaternary base) was dissolved in 0.1 *M* NaH_2PO_4 (pH 4.0) to a concentration of 5 mg/ml and was stored at −20°C as stock solution. Working solutions were prepared as for rocuronium. Stock solution (0.6 mg/ml) of the internal standard (3-desacetylvecuronium) was also prepared in ethanol–water (20:1).

2.5. Blood collection and handling

Whole blood (5 ml) was collected in tubes containing lithium heparin and kept on ice; centrifugation (3000 *g* for 10 min) was carried out within 30 min. Hydrolysis of rocuronium was prevented by adding 200 μl of 1 *M* NaH_2PO_4 per ml of plasma and samples were stored at −20°C until analysed. This is the standard procedure for preventing rocuronium degradation after blood collection [2].

2.6. Plasma extraction procedures

Liquid–liquid ion pair extraction was used with plasma samples. A 1-ml volume of plasma was acidified with 0.2 ml of 1 *M* sodium dihydrogen phosphate and 1.5 ml of saturated potassium iodide (12.8 g potassium iodide and 58.5 mg sodium chloride in 10 ml water, pH 4.0) was added followed with 7 ml of dichloromethane. After a 30-min extraction on a rotary mixer and centrifugation at 3000 *g* for 15 min, the upper dichloromethane phase was transferred to a conical glass tube and the organic phase evaporated under a gentle stream of nitrogen at room temperature. The residue was dissolved in 50 μl of acetone and 2 μl of this was injected into the column.

2.7. Standard curve

Standard curves were generated by spiking drug-free plasma with four concentrations of rocuronium (87, 435, 870, 4345 ng/ml) and 17-desacetylrocuronium (860, 4296, 8592, 42 960 ng/ml) plus internal standard (3-desacetylvecuronium, 600 ng/ml). Drug-free plasma was either frozen plasma from a hospital blood bank or baseline plasma sample taken from a patient before neuromuscular blocker administration. All standard curve samples were subjected to the same extraction procedure as patient samples.

2.8. Extraction efficiency

Extraction efficiency was estimated by adding known concentrations of rocuronium (87 to 4345 ng/ml) or 17-desacetylrocuronium (4296 and 42 960 ng/ml) and 600 ng of internal standard to one set of plasma samples before extraction (set A) while to another set of plasma, internal standard was added before extraction but rocuronium and metabolite were added after extraction but before dichloromethane evaporation (set B). Extraction efficiency was then estimated by comparing rocuronium (or rocuronium metabolite) to internal standard peak area ratios between sets A and B.

Recovery of rocuronium from plasma using plastic or glass centrifuge tubes was also evaluated to rule out adsorption during extraction. Rocuronium was added to two sets of either glass or plastic tubes ($n=3$ each) together with 1 ml of drug-free plasma. The samples were then subjected to the extraction procedure; after centrifugation, the dichloromethane phase was transferred to a glass conical tube to which internal standard was added. The solvent was then evaporated under nitrogen at room temperature. Comparisons of the peak area ratios of rocuronium to

I.S. in the two sets of tubes were made. A similar comparison was made when extractions were carried out in either unsilanised or silanised glass tubes to assess adsorption specifically to glass.

2.9. Assay validation

Intra-day precision was assessed by assaying three sets of unknown samples in triplicate; each set was spiked with different rocuronium (435, 870 and 4345 ng/ml) and 17-desacetylrocuronium (4296, 8592 and 42 960 ng/ml) concentrations. The inter-day variability was calculated by analysing plasma spiked with different analyte concentrations over a 1-week period. Intra- and inter-day precision were expressed as relative standard deviation (RSD). Accuracy was assessed by spiking drug-free plasma and assaying the samples so that the analyst had no knowledge of the spiked concentration. Accuracy (%) was expressed as the assayed over the spiked analyte concentration.

3. Results and discussion

3.1. Mass spectra

Thermal decomposition of the analytes in the mass spectrometer meant that mass spectra of the fragments only were obtained. Base peaks at m/z of 413 for rocuronium, 236 and 447 for rocuronium metabolite and 425 for the I.S. were used for quantifying the analytes using the SIM technique (Fig. 2). Monitoring of these specific ions yielded high sensitivity especially for rocuronium and no interference from endogenous compounds.

Representative chromatograms of extracts of blank plasma spiked with I.S. only, blank plasma spiked with rocuronium, rocuronium metabolite and I.S. and a patient plasma sample are shown in Fig. 3. Rocuronium metabolite, rocuronium and I.S. retention times were 14.8, 15.7 and 16.8 min, respectively. No interfering peaks from endogenous substances or from drugs used for induction and/or maintenance of anaesthesia appeared in the chromatogram when SIM was used. The putative metabolite of rocuronium, 17-desacetylrocuronium, was not detected in patient plasma samples.

3.2. Extraction efficiency

Recovery could only be reliably estimated by adding the analytes to dichloromethane extracts of blank plasma. Otherwise peak areas or heights from extracted samples were much higher than those from unextracted samples leading to unexpectedly high extraction efficiency. This was consistent with previous results with other neuromuscular blockers like vecuronium and pancuronium [4]. A mean recovery of ~75% (Table 1) compared with previously reported recovery of only 48% was obtained using this technique. A similar extraction method except that 1 ml of plasma and 1.5 ml of saturated potassium iodide was used in the current assay whereas 0.5 ml of iodide buffer was used previously with 0.25 ml of plasma. Another difference between this assay and the previously reported method was that sodium chloride was added to the potassium iodide ion pair buffer to optimise the partitioning of the drug–iodide ion pair into the organic phase. Previously, 5 ml of dichloromethane was used with evaporation at 60°C whereas here 7 ml of dichloromethane was used and evaporated at room temperature. A more favourable ratio of iodide ion pair agent and organic solvent and evaporation at room temperature probably also contributed to higher extraction efficiency in this assay. The extraction efficiency was similar from plastic or glass tubes and silanisation of glass did not alter extraction efficiency. Rocuronium metabolite extraction efficiency was much lower at approximately 50% (Table 1), presumably reflecting the more polar nature of this metabolite even after iodide ion pair formation.

3.3. Linearity

The calibration curve was linear from 87 to 4345 ng/ml for rocuronium and 860 to 42 960 ng/ml for 17-desacetylrocuronium. Slopes of the standard curves from different patients were the same as those from blood bank plasma. Regression equation between peak area ratio of analyte/I.S. and concentration was $y=3.2(\pm 0.15)x+0.49(\pm 0.04)$ for

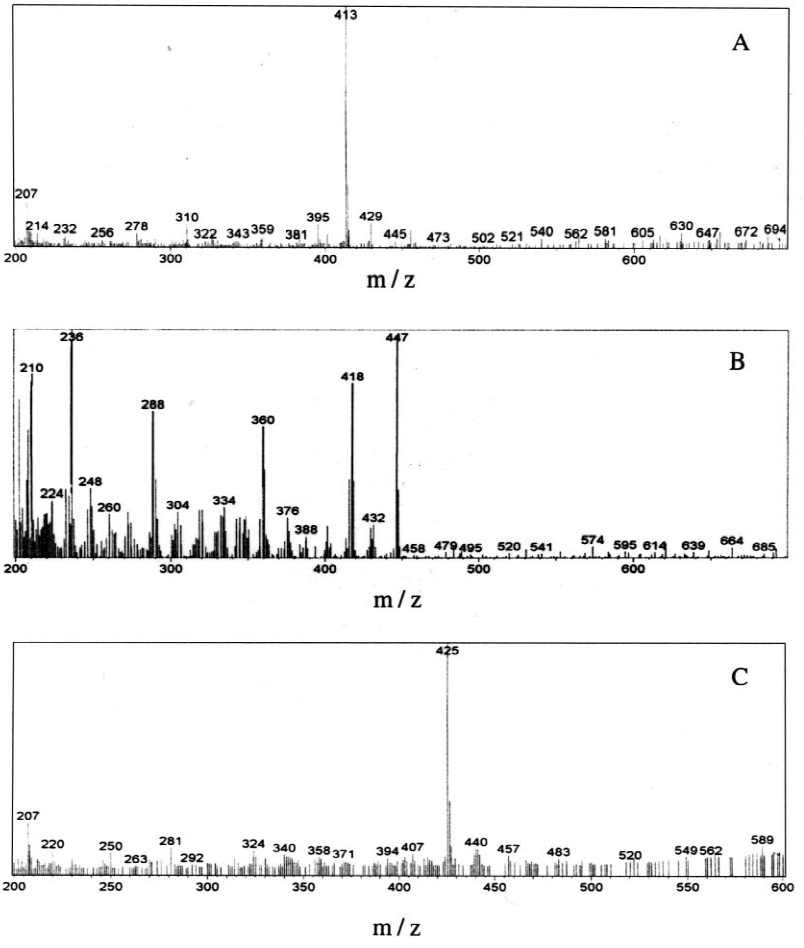


Fig. 2. Mass spectra of rocuronium (A), 17-desacetylrocuronium (B) and 3-desacetylvecuronium, I.S. (C).

rocuronium and $y=0.094(\pm 0.007)x+0.29(\pm 0.025)$ for rocuronium metabolite.

3.4. Sensitivity

Using the SIM technique with m/z 413 as the target ion for rocuronium ~2 ng of rocuronium could be detected on column. The limit of quantification for rocuronium in plasma was 26 ng/ml. This method is thus suitable for pharmacokinetic studies in patients receiving rocuronium during anaesthesia. However, the sensitivity for 17-desacetylrocuronium was not as good as for the parent compound, the lowest amount of metabolite detectable on-column was higher at ~100 ng. Although no degradation products were observed, it is speculated that the high temperature (300°C) in the GC injection port might have led to degradation of the metabolite resulting in lack of adequate sensitivity for 17-desacetylrocuronium. This speculation is consistent with a previous suggestion that derivatisation of rocuronium (and its metabolite) confers thermal stability during GC [5].

3.5. Assay validation

Intra-day precision (RSD) for rocuronium ranged from 1.3 to 7.9% while that for 17-desacetylrocuronium was approximately 10% (Table 2). Inter-day variability was somewhat higher but gener-

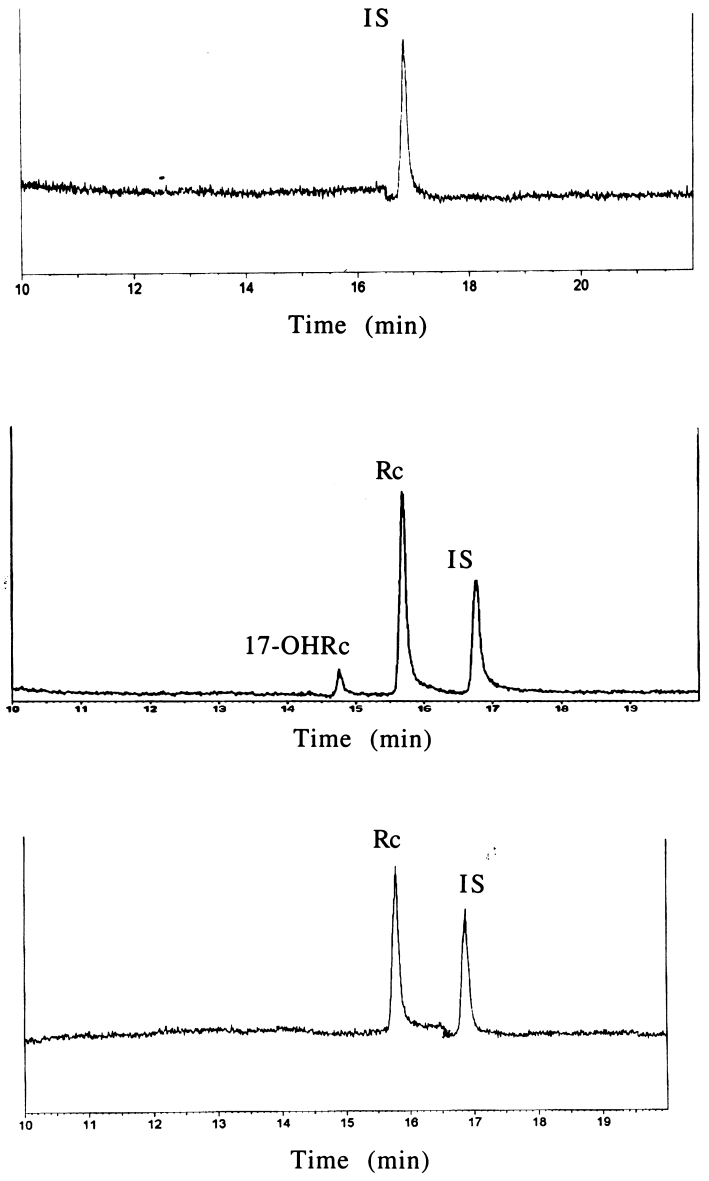


Fig. 3. Chromatograms of extracts of blank human plasma spiked with I.S. only (upper), human plasma spiked with rocuronium (Rc), rocuronium metabolite (17-OHRc) and I.S. (middle) and patient plasma containing rocuronium and spiked with I.S. (bottom). Generated using selected ion monitoring (SIM) mode.

ally less than 15%. Accuracy of assayed samples is presented in Table 3; mean deviation from spiked concentrations was less than 7% for both rocuronium and its metabolite.

4. Clinical applicability of the assay

This assay was used to quantify rocuronium in a patient undergoing liver transplantation surgery and

Table 1
Recovery of rocuronium and 17-desacetylrocuronium from human plasma as iodide ion pair and liquid–liquid extraction

Rocuronium (ng/ml)	Recovery (%)	17-Desacetylrocuronium (ng/ml)	Recovery (%)
87	92±4.0	4296	65±13.6
435	77±7.7	8592	42±10.0
870	76±0.7	42 960	41±5.7
4345	65±6.8	–	–

Results are mean±SD, $n=3$ for each analyte at each concentration.

Table 2
Intra- and inter-assay variability for rocuronium and 17-desacetylrocuronium from human plasma

Concentration (ng/ml)	Intra-assay		Inter-assay	
	Analyte/I.S. PAR	RSD (%)	Analyte/I.S. PAR	RSD (%)
Rocuronium				
435	1.98±0.08	4.0	2.28±0.40	17.5
870	4.80±0.38	7.9	4.97±0.47	9.5
4345	26.71±0.36	1.3	28.18±1.44	5.1
17-Desacetylrocuronium				
4296	0.69±0.06	8.7	0.63±0.09	14.3
8592	1.48±0.14	9.5	1.62±0.21	13.0
42 960	11.01±1.14	10.0	10.43±0.95	9.1

Results are mean±SD, $n=3$ for each analyte at each concentration.

in another patient undergoing general elective surgery. Patient blood samples were kept on ice until centrifugation within 30 min of collection and plasma was acidified to pH 5.5 to prevent rocuronium degradation. Results obtained in one liver transplant patient are presented in Table 4, the patient was given a constant-rate (0.17 mg/kg/h) infusion of rocuronium throughout surgery and blood samples were taken at 30-min intervals until completion of the transplantation. Rocuronium concentrations did not differ between the various periods of liver transplantation but the concentration increased markedly at the beginning of the an-hepatic phase then declined. Rocuronium plasma concentrations in another patient undergoing general elective surgery and receiving an i.v. bolus dose of 0.39 mg/kg of rocuronium is shown in Fig. 4. Concentrations declined in a biexponential manner with an elimination half-life of 21 min. Rocuronium metabolite was not detected in either patient reflecting either the inability of this assay to detect low concentrations of the metabolite or rapid elimination of the metabolite. Another pharmacokinetic study reported that rocuronium metabolite concentrations were negli-

Table 3
Accuracy of rocuronium and 17-desacetylrocuronium assay from human plasma

Spiked concentration (ng/ml)	Accuracy (%)	RSD (%)
Rocuronium		
435	94±6.1	6.5
870	96±7.5	7.8
4345	92±10.1	10.9
17-Desacetylrocuronium		
4296	107±3.0	2.8
8592	90±8.5	9.4
42 960	103±5.2	5.0

Results are mean±SD, $n=3$ for each analyte at each concentration, 600 ng of I.S. was added.

Table 4
Rocuronium plasma concentrations during different phases of liver transplantation surgery in a patient receiving a constant-rate (0.17 mg/kg/h) infusion of rocuronium

	Rocuronium concentration (ng/ml)		
	Pre-an-hepatic	An-hepatic	Re-perfusion
	1758	2121	1239
	1594	1244	1191
	1707	1382	1456
	1634	1380	1700
	1674		1133
	1581		1263
	1305		
Mean±SD	1608±148	1532±398	1330±211
RSD (%)	9.2	25.9	15.9

Concentrations were determined at 30 min intervals.

gible in urine and plasma even after a large dose, indicating that rocuronium is eliminated predominantly unchanged [5].

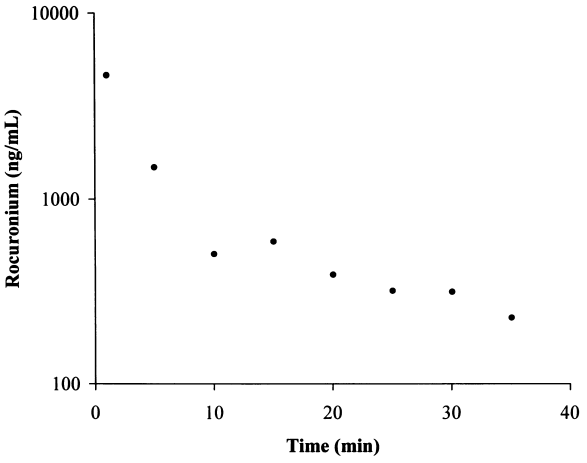


Fig. 4. Plasma concentrations of rocuronium after bolus i.v. administration of 0.39 mg/kg in a patient undergoing general elective surgery.

5. Conclusions

The assay developed here provided sensitive and highly selective determination of the unchanged rocuronium in human plasma. This method also permitted simultaneous determination of its putative metabolite, 17-desacetylrocuronium, but the high detection limit for the metabolite limited the practical application of this method in pharmacokinetic studies of the metabolite. The assay was used successfully in patient pharmacokinetic studies and is a time-saving alternative to published gas or liquid chromatographic methods for assaying rocuronium.

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