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# A sensitive radioimmunoassay, combined with solid-phase extraction, for the sub-nanogram per ml determination of ondansetron in human plasma

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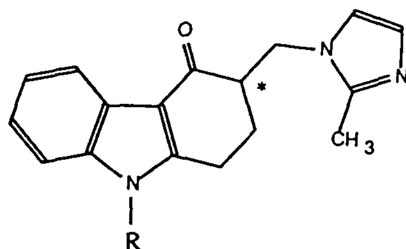
**Abstract**: The development of a radioimmunoassay, incorporating solid-phase sample extraction, suitable for the sub-nanogram per ml determination of ondansetron base in human plasma is described. The antiserum was raised in Soay sheep following primary and booster immunizations with an immunogen prepared by conjugating 9-(carboxypropyl)-ondansetron to bovine thyroglobulin. The radioligand consisted of ondansetron specifically tritium-labelled on the N-methyl group of the indole moiety. The solid-phase extraction method, using a cyanopropyl sorbent, was introduced to remove cross-reacting metabolites and to enhance assay sensitivity. The calibration range is 0.05–2.40 ng $ml^{-1}$ using a 1 ml sample of human plasma; inter- and intra-assay bias and precision are $<\pm 13\%$ and $<10\%$ over this concentration range, respectively. The assay drift, measured as the difference in concentration values for quality control samples assayed immediately before and after the sequence of test plasma samples, is $<\pm 10\%$ for run sizes of up to 54 samples.

**Keywords**: *Ondansetron; plasma; solid-phase extraction; radioimmunoassay.*

## Introduction

Ondansetron (Fig. 1) is a potent and highly selective 5-hydroxytryptamine$_3$ (5-$HT_3$) receptor antagonist that, as ondansetron hydrochloride dihydrate, is being used successfully for the control of emesis in patients undergoing cancer therapy [1–3]. Ondansetron is also effective against post-operative nausea and vomiting [4], and is being investigated for the treatment of CNS indications [5].




(* *asymmetric centre)*

**Figure 1**
Chemical structures of: ondansetron [R, $CH_3$], tritium-labelled ondansetron [R, $C^3H_3$] and the 9-(carboxypropyl)ondansetron hapten [R, $(CH_2)_3COOH$] employed for the production of the immunogen.

Ondansetron possesses one asymmetric centre and has been developed as the racemate. A validated analytical method is available for the antiemesis programme which employs HPLC–UV for the determination of circulating levels of the drug in human plasma [6]. However, the lower limit of quantification is 1 ng $ml^{-1}$ which is not sufficiently sensitive for pharmacokinetic studies proposed in the further development of the drug. Therefore, a radioimmunoassay (RIA) combined with solid-phase extraction (SPE) has been developed to enable the determination of the sub-nanogram levels of ondansetron base anticipated in samples of human plasma from these new investigations.

This paper describes the development of an RIA for the direct determination of ondansetron base in samples of human plasma, the detection of interfering cross-reacting metabolites and the subsequent validation of a method employing solid-phase extraction (SPE–RIA) to selectively remove these metabolites. This latter procedure also further enhanced assay sensitivity through concentration of the sample.

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## Experimental

### *Chemicals*

Chemicals were obtained from several sources. Buffer salts (analytical grade) were purchased from BDH (Poole, Dorset, UK); acetonitrile and methanol (HPLC grade) and propan-2-ol (HPLC grade) were supplied by Rathburn (Walkerburn, Scotland, UK) and Fisons (FSL), respectively. Ondansetron (as the hydrochloride dihydrate), 9-(carboxypropyl)ondansetron hapten, ondansetron enantiomers (Fig. 1) and potential metabolites (Fig. 2) used for the specificity studies were all synthesized by the Chemistry Division, Glaxo Group Research (Ware, Herts, UK). Methyl-($^3H_3$)-iodide was supplied by Amersham International. Most other chemicals used for the preparation of the immunogen and radioligand were obtained from Aldrich; the exceptions were bovine thyroglobulin and Freund's complete adjuvant which were from Sigma. The incomplete non-ulcerative Freund's adjuvant was obtained from Guildhay Antisera (Guildford, UK).

Deionized water (>14 MΩ cm) was obtained from an Elga Elgastat Spectrum system.

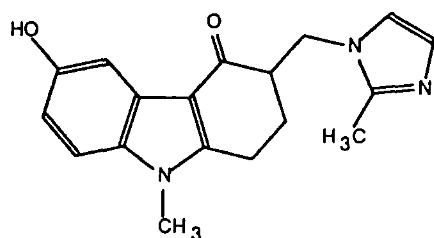
Picafluor 30 liquid scintillation cocktail was obtained from Packard Inc. (Downes Grove, IL, USA).

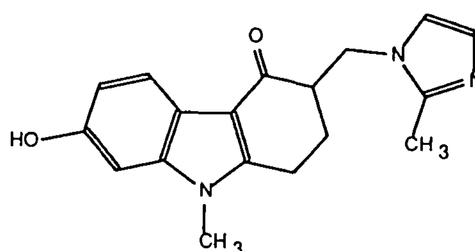
### *Preparation of radioimmunoassay working solutions*

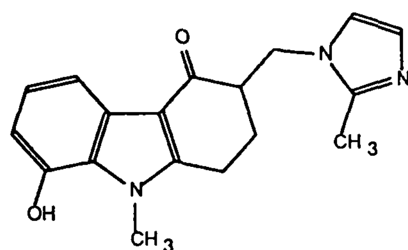
The radioimmunoassay (RIA) diluent consisted of gelatin–phosphate buffered saline solution (pH 7.4): 0.62% (m/v) disodium hydrogen orthophosphate, 0.148% (m/v) sodium dihydrogen orthophosphate, 0.6% (m/v) sodium chloride, 0.05% (m/v) sodium azide and 0.1% (m/v) gelatin (Croda Colloids, Bedford, UK). The solution containing 5% (m/v) bovine serum albumin (BSA, Sigma fraction V; Sigma, Poole, Dorset, UK) was prepared by mixing equal volumes of assay diluent and a stock solution containing 10% (m/v) BSA and 0.05% (m/v) sodium azide. The latter solution could be stored at 4°C in the dark for up to 1 month. The assay diluent and 5% (m/v) BSA solutions were replaced weekly.

Working solutions of the ondansetron radioligand and antiserum (described below) were prepared on each day of analysis. The former was prepared by adding approximately 25 μl of the stock solution to 20 ml of assay diluent; 50 μl of this dilution had a radioactive concentration of approximately 333 Bq (20,000 dpm). The latter was prepared by diluting the undiluted antiserum 1/23,000 in assay diluent.

Primary stock standard solutions of 20% (v/v) methanol containing 1 mg ondansetron



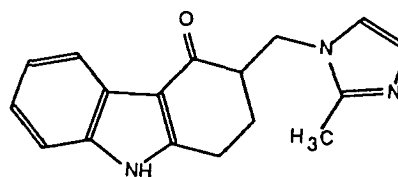




**Figure 2**
Chemical structures of the proposed ondansetron metabolites.

base $ml^{-1}$ were prepared monthly; working standard solutions were prepared, on each day of analysis, from this stock by dilution in assay diluent. All solutions containing ondansetron were kept at 4°C and protected from light prior to use.

Phase-separation of antibody-bound and free (unbound) radioligand was performed using a 0.1% (m/v) dextran T70 (Pharmacia, Uppsala, Sweden) coated 1.0% (m/v) charcoal (hydrochloric acid washed; Sigma) suspension produced in assay diluent. The suspension was stirred for 15 min and then stored at 4°C for at least 2 h prior to resuspension and use in the immunoassay. This reagent was used within 24 h of production.

*Apparatus*

Liquid scintillation counting was performed by means of a Denly Delta counter interfaced to an Olivetti M24 personal computer running the RIACALC II data-acquisition and reduction program (Wallac). The four parameter logistic (4PL) algorithm within RIACALC was employed to convert radioactivity measurements, expressed as counts per minute (cpm), into concentration values (ng $ml^{-1}$).

Solid-phase extraction was performed using 1 ml Bond-Elut cartridges containing 100 mg of cyanopropyl sorbent (Jones Chromatography) and automated by means of a 50- or 150-position vacuum box [7]. The final sample extract was evaporated to dryness in a Savant Speed Vacuum Concentrator.

Purification of the ondansetron radioligand by means of HPLC was performed using a 3 μm Spherisorb $SiO_2$ (150 × 4.6 mm i.d.) column, Gilson model 303 pump, Holochrome UV detector and a N2 *x/t* chart recorder. The chromatographic conditions were: column, 3 μm Spherisorb $SiO_2$ (150 × 4.6 mm i.d.); mobile phase, 0.1% (v/v) triethylamine in distilled ethanol; flow rate, 0.6 ml $min^{-1}$; detection wavelength, 303 nm. Sample injections were made using a Rheodyne model 7125 syringe loading valve equipped with a 500 μl sample loop. (A 500 μl loop was selected to minimize any contamination of the valve's overflow lines that might have occurred if a loop of a similar size to the injection volume had been used.)

*Blood-sample collection*

Blood samples, for the preparation of a normal human plasma pool (control plasma), specificity studies and for the method cross-validation with HPLC, were taken from healthy volunteers by venepuncture into lithium heparin anticoagulant. The samples used for the latter investigation had been collected and analysed previously (by means of HPLC) in studies performed during the development of ondansetron for the anti-emesis indication.

All samples were stored at −20°C and assayed within three freeze–thaw cycles; under these conditions ondansetron is known to be stable in plasma (Glaxo Group Research, unpublished results).

**Methods**

*Synthesis of the $^3H$-ondansetron radioligand*

Ondansetron was specifically tritium-labelled using methyl-($^3H_3$) on the nitrogen of the indole moiety (Fig. 1), as follows: a 23.6 μmol (6.59 mg) amount of N-*des*methyl ondansetron base and 35 μmol (0.35 ml of 0.1 M) of tetrabutyl–ammonium fluoride were added to 10 ml of distilled, dry tetrahydrofuran. An aliquot (0.25 ml) of this solution was cooled to −200°C with liquid nitrogen, whereupon 0.118 μmol (17.4 μg) of methyl-($^3H_3$)-iodide (370 MBq, specific activity 3.15 TBq $mmol^{-1}$) in 1 ml toluene was added, with constant cooling, using a vacuum transfer technique. The temperature of this reaction mixture was raised to approximately 22°C (ambient) and stirred for 3.25 h.

The reaction mixture was then azeotroped with distilled ethanol (3 × 8 ml) to remove the toluene; care was taken not to allow the solution to evaporate to dryness. The final ethanol solution (approximately 1 ml) was purified further by means of semi-preparative HPLC (using 10 injections of 100 μl).

The component eluting at 6.7 min following each of the 10 injections was collected, pooled and evaporated to a volume of approximately 3 ml. This final solution, containing the purified radioligand, was diluted to 50 ml with ethanol and stored in the dark at −20°C prior to use in the RIA.

The radioactive concentration of the radioligand solution was 5.84 MBq $ml^{-1}$ and its radiochemical purity was determined, by means of HPLC, to be greater than 99% $^3H$-ondansetron base.

*Antiserum production*

*Preparation of the immunogen.* An immunogen could not be prepared using the parent molecule because ondansetron does not possess suitable functional groups for direct conjugation to a protein. Therefore, a 9-(carboxypropyl) derivative of ondansetron (Fig. 1) was selected as the hapten and conjugated to bovine thyroglobulin through the carboxylic acid moiety using the mixed anhydride reaction scheme.

The hapten (49.94 μmol, 18.25 mg), tributylamine (58.76 μmol, 14 μl) and isobutyl chloroformate (100.21 μmol, 13 μl) were added sequentially to 1 ml cooled (4°C), dry dimethylformamide (DMF). This continually cooled reaction mixture was stirred, by means of a small magnetic stirring bar, for approximately 30 min. The solution was then transferred dropwise to a similarly cooled solution containing 127.3 nmol of bovine thyroglobulin (84 mg) in 4 ml deionized water. The final solution was stirred on ice for 1 h and then allowed to rise to ambient temperature with continual stirring for a further 1 h. During this period, a precipitate formed in the reaction vial.

The reaction mixture was purified by extensive dialysis against deionized water and finally freeze-dried to constant mass (74 mg of immunogen).

*Antiserum production in sheep.* The immunogen (5 mg per animal) was emulsified with Freund's complete adjuvant and used to immunize, by the intramuscular and subcutaneous routes, two young Soay ewes. The animals were subsequently boosted at 4 and 10 months after the primary immunization with the same immunogen (2.5 mg per animal) emulsified in Freund's incomplete, non-ulcerative adjuvant. Test bleeds were taken immediately before and approximately 14 days after each booster immunization and an acceptable antibody titre (1/23,000), corresponding to 50% maximum binding of the radioligand, was obtained in one animal after a second boost. The serum from this bleed was stored at −60°C.

*Direct radioimmunoassay*

Calibration standards were prepared over the concentration range 0.039–10.00 ng ondansetron base per ml of assay diluent ($n$ = 9). In addition, a zero standard was included in each standard series.

Plasma samples estimated to contain greater than 5 ng of ondansetron base $ml^{-1}$ were diluted in assay diluent to bring them within the linear portion of the standard curve.

Duplicate assays were performed in 5 ml plastic tubes (A/F/8702, Sarstedt) for each standard, quality control (QC) or test sample. In each instance, the final incubation volume was 0.5 ml which consisted of: 0.1 ml standard, QC or sample solution, 0.2 ml assay diluent, 0.1 ml control plasma, 0.05 ml working $^3$H-ondansetron radioligand solution and 0.05 ml working antiserum solution. Non-specific binding (NSB) and total activity tubes were included with each batch of analyses. The former contained 0.35 ml of assay diluent, 0.1 ml of control plasma and 0.05 ml of radioligand; the latter contained 0.95 ml assay diluent and 0.05 ml radioligand solution.

The assay tubes were incubated for approximately 20 h (overnight) at 4°C. Subsequently, phase-separation was performed by adding 0.5 ml of cooled (4°C), well-stirred, dextran-coated charcoal suspension. Each of the tubes was vortex-mixed and then centrifuged immediately (3500 rpm, 15 min, 4°C). Finally, 800 μl aliquots of each supernatant were transferred into scintillation vials by a Tecan 5052 robotic sample processor.

Scintillation cocktail (8 ml) was added to each of the vials, which were then capped, shaken, and loaded on to the liquid scintillation counter. The vials were allowed to equilibrate for approximately 3 h and then counted for 5 min.

*Solid-phase extraction–radioimmunoassay (SPE–RIA)*

Solid-phase extraction (SPE), on a cyanopropyl sorbent was employed, prior to RIA, to selectively retain the parent drug and to concentrate the sample. The order of additions to the columns is as follows.

1. 2 × 1 ml methanol
2. 2 × 1 ml fresh elution reagent 1.25%, (v/v) ammonia solution [35% (v/v)] in propan-2-ol
3. 2 × 1 ml deionized water
4. 50 μl 0.5 M HCl immediately followed by 1 ml plasma to enable acid and sample to mix
5. 2 × 1 ml deionized water

6. 2 × 1 ml acetonitrile
7. 2 × 1 ml fresh elution reagent

The extraction was performed on a vacuum extraction box [7]; however, during steps 1–4, the liquids were allowed to pass through the cartridges without the assistance of reduced pressure, and the cartridges were not allowed to dry out. After both steps 5 and 6, a negative pressure of 0.5 bar was applied to dry the cartridges.

The final extracts (step 7) were collected (flow rate, ≈1 drop every 2 s), dried to residue in a Savant vacuum concentrator (≈45°C, 2.5 h) and reconstituted in 300 μl of assay diluent by vortex-mixing (15 min).

These extracts were subjected to duplicate analysis by RIA using a method similar to the direct RIA described above. The only exception was that 0.1 ml of 5% (m/v) BSA-assay diluent solution was added to each tube instead of control plasma. Calibration standards were prepared, in control plasma, over the range 0.0125–2.40 ng ondansetron base $ml^{-1}$ and, together with QC samples, subjected to the same extraction procedure as the test plasma samples.

The SPE–RIA procedure was used for method validation studies and to analyse volunteer plasma samples.

*Validation of the SPE–RIA method*

The SPE–RIA method was validated in accordance with recommendations [8–11] which assess the performance of an assay with regards to its specificity, recovery of drug (when an extraction procedure is used), sensitivity, accuracy and precision. In addition, quality control samples were used to determine the extent of any drift in concentration values from the beginning to the end of a typical sequence of test samples.

*Specificity studies*

(1) *Cross-reactivity*. The specificity of the SPE–RIA method, with regard to metabolites previously identified in urine (N-*des*methyl ondansetron and 6-, 7- and 8-monohydroxylated ondansetron, Fig. 2) and the (*R*) and (*S*) enantiomers of the ondansetron racemate (Fig. 1), was determined by assaying aliquots of control plasma that had been spiked with each of the compounds over the concentration range 0.1–5.0 ng $ml^{-1}$. For comparison, the cross-reactivity was also determined for the same metabolites in unextracted solutions of assay diluent (concentration range, 0.02–100 ng $ml^{-1}$).

(2) *Interference by endogenous plasma components*. Drug-free plasma samples, taken from 20 different volunteers were analysed by the SPE–RIA method to assess assay interference by endogenous material.

*Extraction efficiency*

The extraction efficiency was determined for plasma samples spiked with ondansetron base over a concentration range equivalent to 0.024–30.00 ng loaded on column. Two sets of calibration standards were prepared; the first was produced in control plasma and subjected to SPE treatment as described above, whilst the second was prepared in assay diluent and analysed directly by RIA. The concentration of ondansetron base present in the SPE extracts was determined by reference to the unextracted series of standards.

*Assay accuracy and precision*

The inter- and intra-assay accuracy and precision were determined by analysis of aliquots of control plasma spiked with ondansetron at each of the calibration standard values. The inter-assay investigations were performed over six independent assay runs on identical spiked samples that had been aliquoted and stored at −20°C prior to analysis. Intra-assay performance was assessed by six replicate determinations of the spiked control plasma.

In each instance, the spiked plasma samples and calibration standards were prepared using different batches of control plasma and different 1 mg $ml^{-1}$ stock standard solutions of ondansetron base.

*Determination of assay drift*

Assay drift was investigated by comparing ondansetron concentrations in quality control samples analysed before and after the sequence of test samples in 13 independent assays. The quality control samples were prepared containing 1 and 20 ng ondansetron base $ml^{-1}$ control plasma. The latter samples were assayed diluted 40- and 80-fold in control plasma.

*Cross-validation with an established HPLC–UV method [6]*

One hundred human plasma samples, rep-

resenting five different pharmacokinetic profiles taken from four different volunteers who had been administered 8 mg ondansetron orally, were analysed by the SPE–RIA method. The analyses were performed in five independent assay runs (one profile per run) and the concentrations determined were compared to those obtained by previous analysis using the HPLC–UV method [6].

## Results and Discussion

*Solid-phase extraction–radioimmunoassay (SPE–RIA)*

Initially, a direct radioimmunoassay (RIA) was developed for the determination of ondansetron base in unextracted plasma samples using a normal human plasma pool (control plasma) to match sample and standard

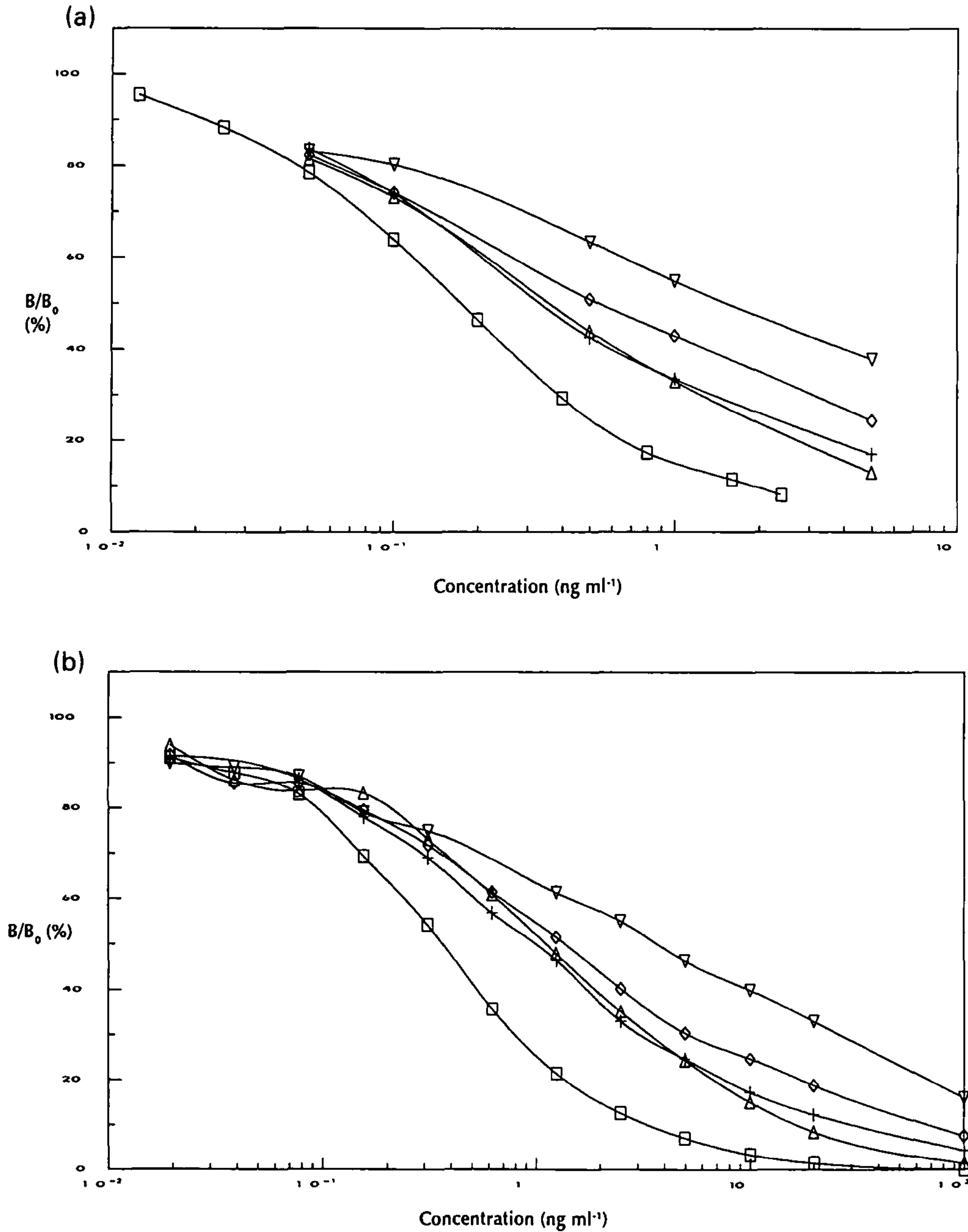



**Figure 3**
Cross-reactivity curves for the proposed ondansetron metabolites, determined by: (a) SPE–RIA, (b) RIA; where: □, ondansetron; △, N-*des*methylondansetron; ▽, 6-hydroxyondansetron; ◇, 7-hydroxyondansetron; +, 8-hydroxyondansetron and (c) by SPE–RIA for the enantiomers of the ondansetron racemate; where: □, ondansetron base; △, (*R*) and ▽, (*S*) enantiomers.

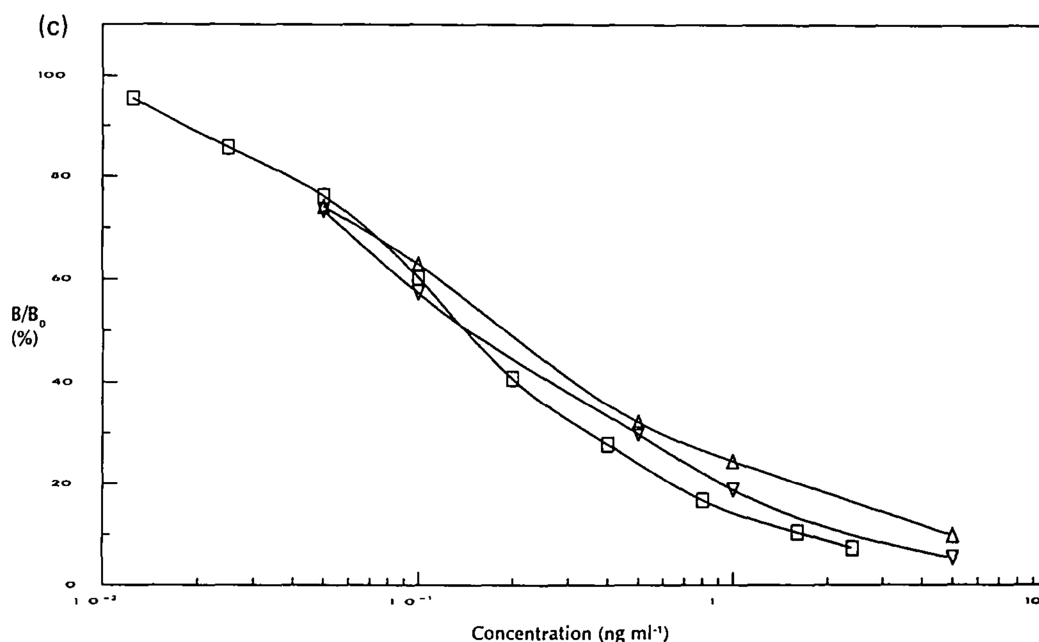



**Figure 3**
Continued.

matrices. This method allowed the sensitive (150 pg ml$^{-1}$) determination of ondansetron base spiked into human plasma samples. However, positive interferences were observed from cross-reacting metabolites present in samples taken from volunteers who had been administered the drug (results are presented below in the description of assay cross-validation with HPLC). In view of this lack of specificity, a sample pre-treatment method was introduced which employed solid-phase extraction on cyanopropyl Bond-Elut cartridges to selectively retain the parent drug, and as a further benefit, to introduce a concentration step to enhance assay sensitivity.

### *Validation of the SPE–RIA method*

*Specificity studies*

(1) *Cross-reactivity.* Cross-reactivity curves (Fig. 3a–c) for the ondansetron enantiomers and proposed metabolites (Figs 1 and 2, respectively) were constructed by plotting antibody binding [Bound/Maximum binding $\mathbf{B_0}$ (%)] against concentration for each compound. The concentrations corresponding to 50% maximum binding (50% $\mathbf{B/B_0}$) were interpolated for each compound and used to calculate the extent of cross-reactivity (Table 1), where:

$$\text{Cross-reactivity (\%)} = \frac{\text{Conc. of ondansetron base at 50\% } \mathbf{B/B_0}}{\text{Conc. of metabolite or enantiomer at 50 \% } \mathbf{B/B_0}} \times 100.$$

Clearly, the values in Table 1 indicate that the metabolites cross-react with the antiserum ranging, for the direct RIA, from 8.9% for 6-hydroxyondansetron base to 33.7% for 8-hydroxyondansetron base. The corresponding values for the SPE–RIA were slightly higher (10.8–50.6%). For both methods, the cross-reactivity values increase as the point of modification nears the position of the indole nitrogen, as the latter is the conjugation site in the hapten–thyroglobulin immunogen used for antiserum production. The higher values determined by SPE–RIA suggest that recovery of the proposed metabolites in the extraction step is relatively greater than that for the parent drug; however, the cross-reactivity profiles are essentially similar for both methods.

Fortunately, the cross-reactivity of these proposed metabolites was found to be of no practical consequence in terms of the specificity of the assay for the analysis of *ex-vivo* plasma samples because these compounds are not present in measurable quantities (<2 ng ml$^{-1}$) in the circulation of man following either oral or intravenous doses of 8 mg ondansetron. In these latter studies, the maximum plasma concentrations of the parent drug were approximately 30 ng ml$^{-1}$ and 105 ng ml$^{-1}$ for each route of administration,

**Table 1**
Cross-reactivity values for the proposed metabolites and enantiomers of ondansetron

| Compound | Cross-reactivity determined by SPE–RIA (%) | RIA (%) |
|---|---|---|
| Ondansetron base | 100.0 | 100.0 |
| N-*des*methylondansetron | 47.8 | 29.1 |
| 6-Hydroxyondansetron | 10.8 | 8.9 |
| 7-Hydroxyondansetron | 31.3 | 22.9 |
| 8-Hydroxyondansetron | 50.6 | 33.7 |
| Ondansetron enantiomers: (*R*) | 79.2 | * |
| (*S*) | 85.2 | * |

* Not determined.

respectively (Glaxo Group Research, unpublished results). However, glucuronide and sulphate conjugates of 8-hydroxyondansetron do occur in significant concentrations and it is these metabolites that are considered to cause the positive interference in the direct RIA. Unfortunately, these compounds were not available for direct cross-reactivity studies; however, their significance in assay specificity is described below (cross-validation with HPLC).

The similar cross-reactivity results observed for each enantiomer indicate that the method is not biased towards either compound.

(2) *Interference by endogenous plasma components.* Twenty different drug-free human plasma samples were assayed and each was found to be free of any interfering components. These results indicate that the method is specific for ondansetron base with respect to endogenous constituents present in human plasma.

*Extraction efficiency*

The recovery of ondansetron base from control plasma spiked with the drug was similar over the concentration range equivalent to 0.023–30.00 ng loaded on column; the mean recovery was 89.2 ± 3.92% (mean ±SD, $n = 16$).

*Assay accuracy, precision, sensitivity and calibration range*

Summary inter- and intra-assay accuracy and precision data for the determination of ondansetron base in spiked samples of control plasma are presented in Table 2.

The accuracy and precision data presented were used to define the lower and upper limits of quantitation for the assay. The lower limit of quantification was set at 0.050 ng ondansetron base $ml^{-1}$ of plasma, where both inter- and intra-assay precision are less than 10% and bias is less than ±13%. At the same level of accuracy and precision, the upper limit of quantification is 2.40 ng $ml^{-1}$; hence, the

**Table 2**
Statistical analysis of inter- and intra-assay accuracy and precision data for ondansetron base in spiked human plasma

| | Inter-assay summary data ($n = 6$)* | | | Intra-assay summary data ($n = 6$)* | | |
|---|---|---|---|---|---|---|
| Authentic conc. (ng $ml^{-1}$) | Mean (ng $ml^{-1}$) | RSD (%) | Bias (%) | Mean (ng $ml^{-1}$) | RSD (%) | Bias (%) |
| 0.0125 | 0.0127 | 44.0 | +1.3 | 0.0198 | 11.7 | +58.7 |
| 0.025 | 0.027 | 24.1 | +9.3 | 0.033 | 21.2 | +32.7 |
| 0.050 | 0.046 | 9.3 | −7.0 | 0.056 | 5.8 | +13.0 |
| 0.100 | 0.102 | 9.1 | +2.2 | 0.102 | 4.6 | +2.0 |
| 0.200 | 0.196 | 4.9 | −1.9 | 0.206 | 2.2 | +2.8 |
| 0.400 | 0.399 | 4.9 | −0.3 | 0.410 | 4.8 | +2.5 |
| 0.800 | 0.807 | 5.4 | +0.8 | 0.836 | 3.5 | +4.5 |
| 1.600 | 1.627 | 7.1 | +1.7 | 1.576 | 2.3 | −1.5 |
| 2.400 | 2.545 | 8.2 | +6.1 | 2.379 | 3.5 | −0.9 |

* Each determination represents the mean of duplicate tubes.

working calibration range is 0.05–2.40 ng ondansetron base $ml^{-1}$ human plasma. A mean calibration graph is presented in Fig. 4.

*Assay drift*

The statistical data for the quality control (QC) samples assayed at the beginning and end of each sequence of samples in 13 independent assays (average run size was 30 samples including QCs) are presented in Table 3. For each QC level, the differences in the mean concentration values are less than ±10% which is within tolerance for anticipated precision and indicates that the method is free from any significant drift in concentration values within a given assay. These results were confirmed in the separate investigation performed to determine intra-assay accuracy and precision where the run size was increased to 54 samples. In this study the drift in concentration was either equal to, or less than, ±10%.

*Cross-validation with an established HPLC–UV method*

One hundred plasma samples, representing five full pharmacokinetic profiles taken from four healthy volunteers who had been administered 8 mg of ondansetron orally, were assayed by direct RIA, SPE–RIA and an established HPLC–UV method [6].

A typical set of pharmacokinetic profiles, obtained by each method for one volunteer, is presented in Fig. 5.

This figure demonstrates that the SPE–RIA and HPLC–UV methods produce virtually superimposable pharmacokinetic profiles,

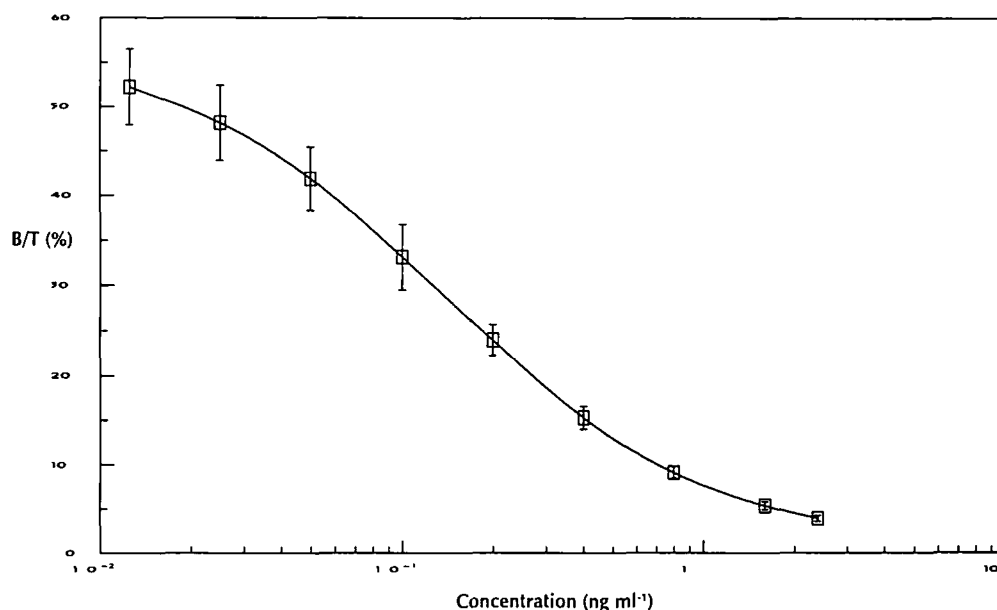



**Figure 4**
Mean inter-assay calibration graph for ondansetron base by SPE–RIA. Each point represents the mean ± the standard deviation for six successive calibrations.

**Table 3**
Statistical analysis of the measured concentrations obtained for ondansetron in QC samples assayed at the beginning and end of each sample sequence

| | Authentic conc. (ng $ml^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | 1.00 | | 0.50† | | 0.25‡ | |
| | beginning | end | beginning | end | beginning | end |
| Mean (ng $ml^{-1}$) | 1.030 | 1.036 | 0.493 | 0.517 | 0.244 | 0.267 |
| SD (ng (ml $^{-1}$) | 0.041 | 0.053 | 0.036 | 0.042 | 0.020 | 0.022 |
| RSD (%) | 3.96 | 5.15 | 7.22 | 8.07 | 8.21 | 8.30 |
| $n$* | 13 | 13 | 13 | 13 | 13 | 13 |
| Drift (%) | | +0.6 | | +4.9 | | +9.4 |

* Each determination represents the mean of duplicate tubes.
†20 ng $ml^{-1}$ QC sample diluted 40-fold in control plasma.
‡20 ng $ml^{-1}$ QC sample diluted 80-fold in control plasma.

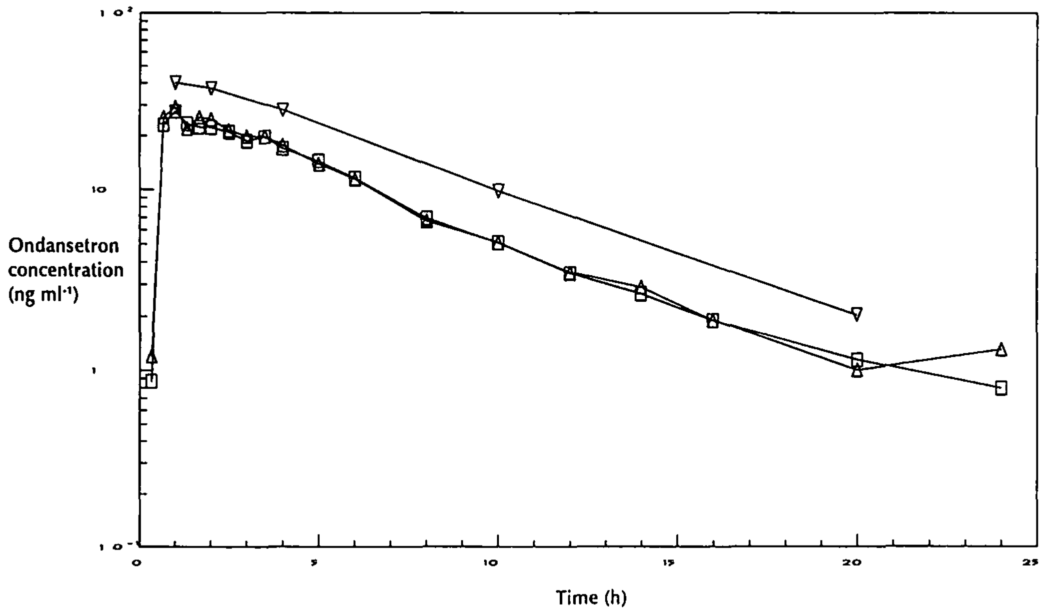



**Figure 5**
Pharmacokinetic profiles for ondansetron base in one volunteer; by: △, HPLC; □, SPE–RIA; and ▽, direct RIA.

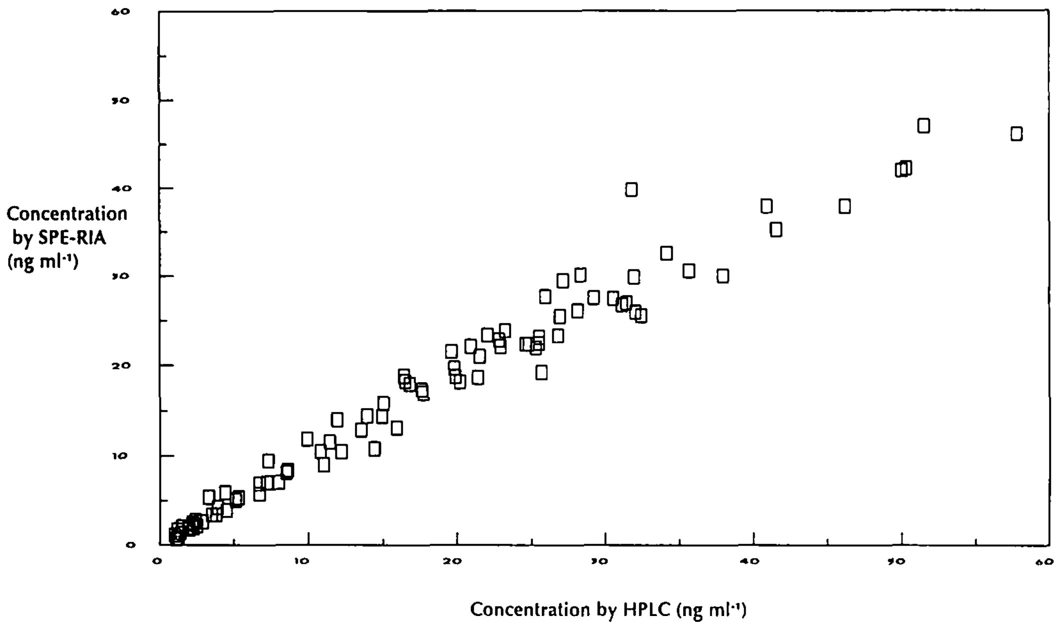



**Figure 6**
Correlation plot of plasma ondansetron concentrations determined by SPE–RIA and HPLC–UV.

whilst the profile obtained by the direct RIA has a marked positive bias compared to the other methods. The anomalous data obtained by the latter method are considered to occur owing to cross-reactivity of the antiserum with metabolites formed by either glucuronidation or sulphation of the 8-hydroxyondansetron metabolite. These conjugates are known to be the major metabolites of ondansetron produced in man [12] (and Glaxo Group Research, unpublished results). The solid-phase extraction method used in the SPE–RIA is selective for the parent drug because the more polar metabolites are considered to elute off the cyanopropyl sorbent during the deionized water wash which is applied to the cartridges prior to elution of the parent drug. Any potentially interfering compounds remaining on the cartridges by non-polar interactions are considered to be eluted by the acetonitrile wash.

The correlation plot (Fig. 6) compares the

data obtained by the SPE–RIA and HPLC–UV methods. Prior to performing regression analysis it was necessary to carry out a $\log_{10}$ transformation on both data sets. This allowed more weight to be applied to the lower concentrations where greater metabolite interference might have been expected and was appropriate in view of the broad range of the concentration values. The least-mean-squares regression equation obtained for the transformed data, assuming equal errors was:

$$y = 0.008 + 0.975\,x.$$

(95% Confidence intervals for the intercept and slope are −0.029–0.045 and 0.94–1.006, respectively.)

The 95% confidence intervals indicate that the intercept and slope pass through zero and unity, respectively. Therefore, there is no fixed or relative bias between the SPE–RIA and HPLC–UV methods and results obtained by the two techniques are equivalent.

## Conclusions

A radioimmunoassay, incorporating a solid-phase sample extraction step, has been developed and fully evaluated for the determination of ondansetron in human plasma. The method is sensitive (lower limit of quantification, 50 pg $ml^{-1}$), functionally specific, precise, accurate and has been fully cross-validated against an established HPLC–UV method.

The method is 20 times more sensitive than the HPLC–UV method and is potentially suitable for the determination of ondansetron in samples from low-dose kinetic studies.

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