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Determination of the enantiomers of ifosfamide and its 2- and 3-N-dechloroethylated metabolites in plasma and urine using enantioselective gas chromatography with mass spectrometric detection

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ABSTRACT

A rapid, sensitive, enantioselective gas chromatographic method has been developed for the quantitation of the enantiomers of ifosfamide (IFF) and its 2- and 3-dechloroethylated metabolites (2-DCE-IFF and 3-DCE-IFF) in human and animal plasma and human urine. IFF and the two dechloroethylated metabolites were extracted into chloroform, enantioselectively resolved by gas chromatography on a chiral stationary phase based upon heptakis(2,6-di-O-methyl-3-O-pentyl)-β-cyclodextrin and quantitated using mass-selective detection with selected-ion monitoring. The limits of quantitation for the enantiomers of IFF, 2-DCE-IFF and 3-DCE-IFF in plasma were 250 and 500 ng/ml respectively. In urine, the limits of quantitation for the enantiomers of IFF, 2-DCE-IFF and 3-DCE-IFF were 500 ng/ml. The method can detect concentrations as low as 250 ng/ml of each enantiomer of 2- and 3-DCE-IFF in plasma and urine. The intra- and inter-day coefficients of variation for this method were with one exception less than 8%. The assay was validated for enantioselective pharmacokinetic studies in humans and rats and is the first reported enantioselective assay for the measurement of the enantiomers of 2- and 3-DCE-IFF in plasma.

INTRODUCTION

Ifosfamide (IFF, Fig. 1) is an oxazaphosphorine alkylating agent related to cyclofosfamide (CP) which is used as an antineoplastic drug. IFF is a prodrug which is converted by P_{450} mixed-function oxidases to 4-hydroxyifosfamide (4-OH-IFF) [1]. This metabolite is transported into the cells where it is converted to the cytostatically active isofosforamide mustard [1].

IFF can also be oxidatively metabolized at the chloroethyl side chains leading to the pharmacologically inactive 2-dechloroethyl-IFF (2-DCE-IFF, Fig. 1) and 3-dechloroethyl-IFF (3-DCE-IFF, Fig. 1). Dechloroethylation accounts for up

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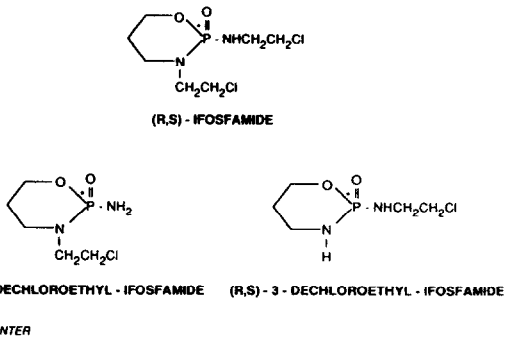

Fig. 1. Structures of ifosfamide and its dechloroethylated metabolites.

to 48% of the administered dose [2] and produces chloroacetaldehyde, a central nervous system (CNS) toxin. Increased plasma levels of chloroacetaldehyde have been associated with observed CNS toxicity [3].

IFF is a chiral molecule which is administered as a racemic mixture in clinical practice and exists in two enantiomeric forms, (*R*)-IFF and (*S*)-IFF. Initial work on the metabolism of this drug suggested a stereospecific difference in the metabolism of the enantiomers of IFF [4]. Using ^{31}P NMR analysis and a chiral shift reagent, the investigators demonstrated that urine samples from two patients who had received rac-IFF were enriched with (*R*)-IFF and (*S*)-2-DCE-IFF; the production of the dechloroethylated metabolites from (*S*)-IFF was 2.7–6.7 fold higher than that from the corresponding (*R*)-IFF. These results have been confirmed by subsequent studies [2,5–7].

Enantioselective HPLC and GC separations of the enantiomers of IFF and its dechloroethylated metabolites have been achieved using chiral stationary phases (CSPs). The HPLC approaches involved CSPs composed of either polyacrylamide [8] or derivatized cellulose [9]. These methods could be used for the preparative separation of IFF enantiomers but were not sensitive enough for routine use in clinical and pharmacokinetic studies.

Two enantioselective GC assays utilizing a CSP based upon polymeric L-valine-*tert*-butylamide (Chiralsil-Val) have also been reported [2,10]; one employed flame-ionization detection (FID) [2] and the other mass-selective detection (MSD) [10]. The latter method was developed and validated in this laboratory and used in pharmacokinetic studies in animals [11] and children [7]. However, under the experimental conditions, the CSP had a relatively short life-time, suffered from column-to-column irreproducibility and constantly bled into the ion source. As a result, studies utilizing this approach were expensive and involved unacceptable levels of down-time.

In order to overcome these problems, we searched for a new GC-CSP with increased stability and reproducibility. The solution was a phase developed by König *et al.* [12] which is based upon heptakis(2,6-di-O-methyl-3-O-pentyl)-β-cyclodextrin (2,6-Me-3-Pe-β-CD-CSP). The present paper describes an enantioselective assay for the determination of the enantiomers of IFF and its dechloroethylated metabolites on this CSP using MSD. Under the assay conditions, the 2,6-Me-3-Pe-β-CD-CSP was stable, had better chromatographic properties than the Chiralsil-Val-CSP and did not bleed into the ion source. The method has been validated for use with human plasma and urine and rat plasma.

EXPERIMENTAL

Chemicals

rac-Ifosfamide was supplied by Bristol-Myers Canada (Belleville, Ont., Canada) and individual enantiomers were prepared by enantioselective HPLC [8]. rac-2-DCE-IFF, (*S*)-2-DCE-IFF, rac-3-DCE-IFF and (*S*)-3-DCE-IFF were gifts from G. Blaschke (University of Münster, Münster, Germany). Cyclophosphamide used as internal standard (I.S.) was obtained from Sigma (St. Louis, MO, USA). Analytical grade methanol and chloroform were obtained from Anachemia (Montréal, Que., Canada).

Apparatus

GC–MS analyses were performed with a Varian 3400 GC equipped with a Finnigan A 200S GC autosampler operating in the splitless mode. The mass spectrometer was a Finnigan MAT Model Incos 50 (Finnigan Nat. Corp., San Jose, CA, USA) operating in the electron-impact and

TABLE I

RESULTS FROM THE ENANTIOSELECTIVE GAS CHROMATOGRAPHIC SEPARATION OF THE ENANTIOMERS OF IFOSFAMIDE (IFF) AND ITS 2- AND 3-DECHLOROETHYLATED METABOLITES (2-DCE-IFF AND 3-DCE-IFF) ON A HEPTAKIS(2,6-DI-O-METHYL-3-O-PENTYL)-β-CYCLODEXTRIN CHIRAL STATIONARY PHASE

See text for experimental details.

Compound	Retention time (min)	k'	Enantioselectivity (α)	Enantioselective resolution factor (R_{RS})
(*R*)-IFF	18.17	35.34	1.06	1.67
(*S*)-IFF	19.15	37.30		
(*R*)-2-DCE-IFF	10.35	19.70	1.03	1.11
(*S*)-2-DCE-IFF	10.67	20.34		
(*S*)-3-DCE-IFF[a]	15.35	29.70	1.28	4.30
(*R*)-3-DCE-IFF[a]	19.54	38.08		
(*R*,*S*)-CP	20.62	40.24	ND[b]	ND[b]

[a] (*S*)-3-DCE-IFF is a metabolite of (*R*)-IFF and (*R*)-3-DCE-IFF arises from (*S*)-IFF.
[b] ND = no enantioselectives separation detected.

selected-ion monitoring (SIM) mode. The chromatographic separation was performed with a capillary column (8 m × 0.25 mm I.D., 0.25 μm film thickness) coated with heptakis(2,6-di-O-methyl-3-O-pentyl)-β-cyclodextrin [12]. The dead volume of the column was determined using methanol.

Chromatographic conditions

Chromatography was carried out using a column temperature gradient of 140°C to 180°C at 1°C/min. The other experimental parameters were as follows: injector temperature 175°C, helium pressure of 21 kPa, transfer line temperature 180°C, ion source temperature 180°C, electron ionization at 70 eV. The detection of the enantiomers of IFF, its dechloroethylated metabolites and CP (the I.S.) was performed using selected-ion monitoring; IFF was monitored at 211 *m/z*, DCE-IFF 149 *m/z*, CP 211 *m/z*. The quantitation of the target compounds was accomplished using peak-area ratios of the compounds to the internal standard.

Preparation of standards

Stock solutions of IFF, 2-DCE-IFF, 3-DCE-IFF, and I.S. were prepared by dissolving each pure compound in methanol at a concentration of 1 mg/ml and diluted to make calibration standards. These solutions were stored at −20°C until used.

Sample preparation

Plasma. To 0.1 ml of the plasma samples (human or rat) were added 20 μl of the internal standard solution (CP, 100 μg/ml in methanol) and 3 ml of chloroform. The resulting mixture was vortex-mixed for 1 min and centrifuged at 1000 *g* for 10 min, the aqueous phase discarded, the organic phase transferred to another tube and evaporated to dryness in a Speed-Vac concentrator. The residue was reconstituted in 100 μl of methanol and 1 μl was injected into the gas chromatograph.

Urine. To a 100-μl volume of urine were added 50 μl of aqueous sodium hydroxide (1 *M*) and 20 μl of internal standard solution (CP, 100 μg/ml in methanol) followed by 200 mg of crystalline sodium chloride. The mixture was vortex-mixed with 3 ml of chloroform and centrifuged at 1000 *g* for 10 min. The aqueous phase was discarded, the organic phase transferred to another tube and evaporated to dryness in a Speed-Vac concentrator. The residue was recon-

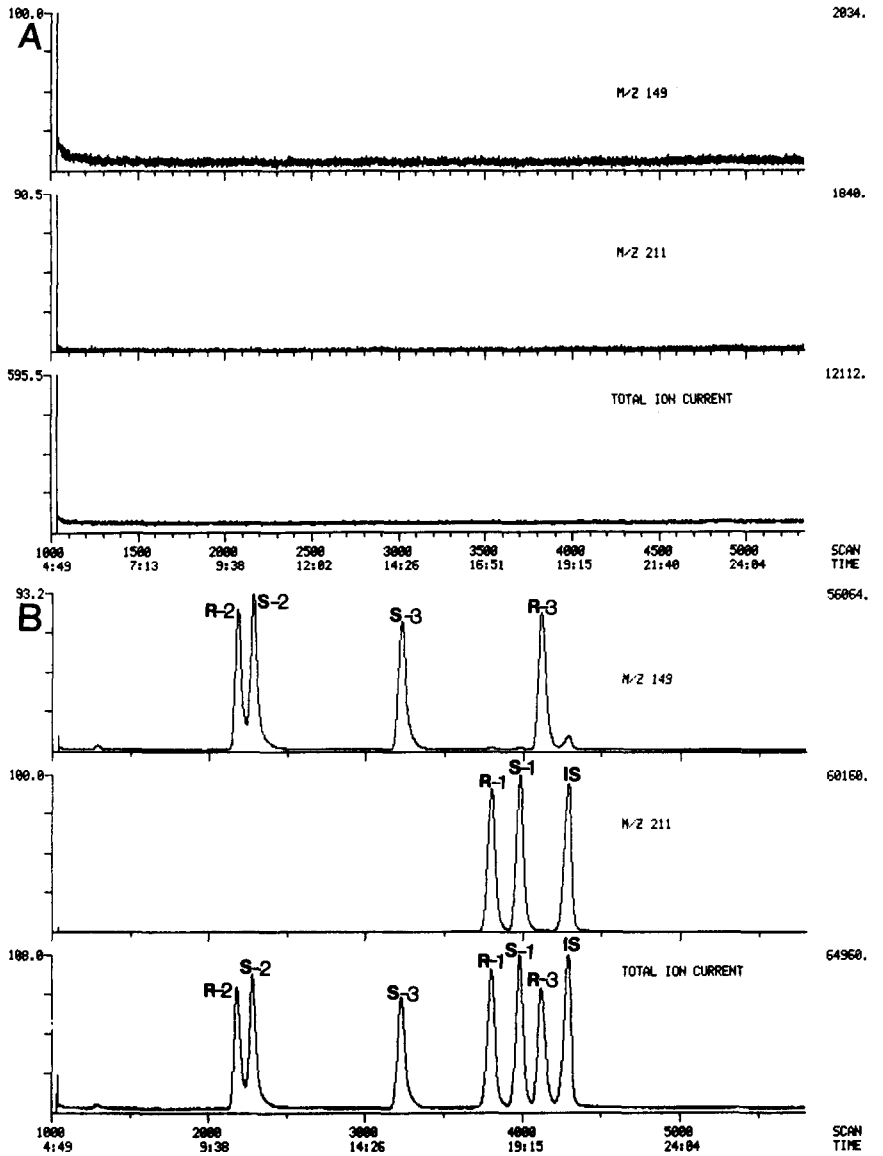

Fig. 2. Typical selected-ion chromatograms and total ion current of the enantioselective separation of the enantiomers of IFF and its dechloroethylated metabolites. (A) Blank plasma; (B) plasma samples spiked with 20 μg/ml each of rac-IFF, rac-2-DCE-IFF and rac-3-DCE-IFF. Where: R-2 = (*R*)-2-DCE-IFF, S-2 = (*S*)-2-DCE-IFF, S-3 = (*S*)-3-DCE-IFF, R-1 = (*R*)-IFF, S-1 = (*S*)-IFF, R-3 = (*R*)-3-DCE-IFF, I.S. = CP. See text for chromatographic conditions.

stituted in 100 μl of methanol and 1 μl was injected into the gas chromatograph.

Validation studies

Plasma. Standard curves were prepared from the stock solutions in drug-free plasma with IFF concentrations of 0.25–75 μg/ml for each enantiomer and 0.25–50 μg/ml for each enantiomer of 2-DCE-IFF and 3-DCE-IFF. The samples were kept at −20°C until analysis. Standard curves were run in triplicate. Inter-day and intra-day studies were performed by spiking drug-free

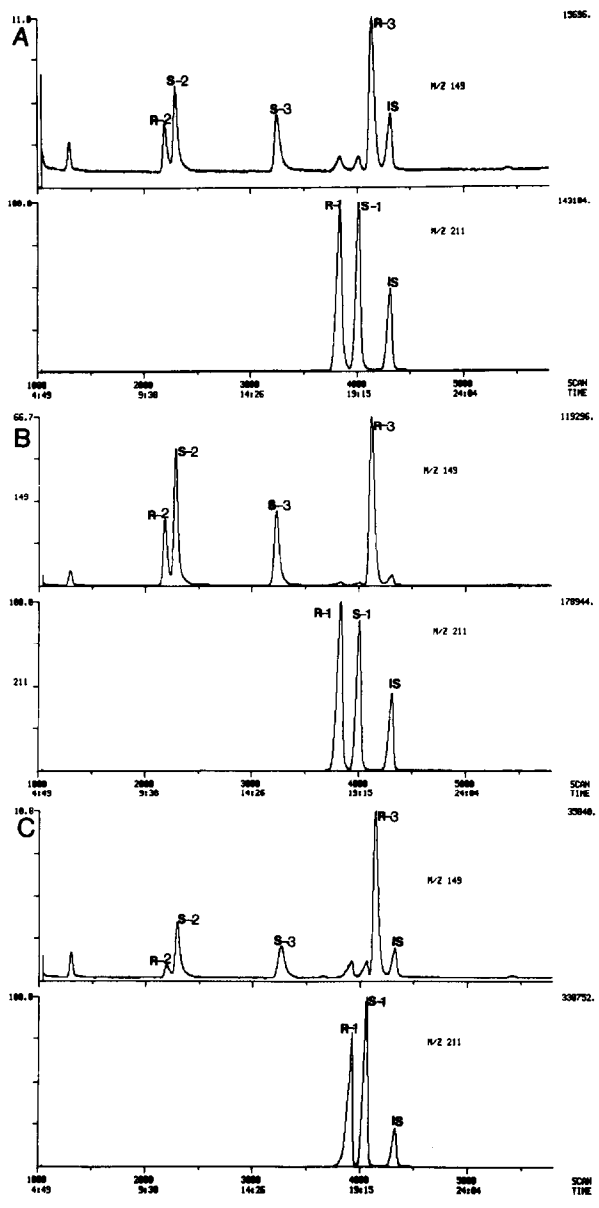

Fig. 3. Selected-ion chromatograms of human plasma and urine samples from a patient treated with rac-IFF (3 g/m^2) and rat plasma after the i.v. administration of 125 mg/kg rac-IFF. (A) Human plasma sample 10 h after the end of a 170 min intravenous infusion; (B) urine samples 18 h after dosing; (C) rat plasma sample 4 h after dosing.

plasma with 2.5, 20 and 60 μg/ml of each enantiomer of IFF and 2.5 and 30 μg/ml of each enantiomer of 2-DCE-IFF and 3-DCE-IFF. Accuracy was investigated by the comparison of measured to expected concentration. The precision is expressed as the percentage deviation from the expected concentration.

Urine. For the urine standard curve, the concentrations of IFF, and 2- and 3-DCE-IFF ranged from 0.5 to 100 μg/ml for each enantiomer. The samples were kept at −20°C until analysis. Standard curves were run in triplicate. Inter-day and intra-day studies were performed by spiking blank urine with rac-IFF, rac-2-DCE-IFF and rac-3-DCE-IFF to produce urine concentrations of 2.5, 30 and 75 μg/ml of each enantiomer. Accuracy was investigated by the comparison of measured to expected concentration. The precision is expressed as the percentage deviation from the expected concentration.

Extraction yield

Recoveries were estimated by comparing the peak-area ratios for each isomer by direct injection of standard samples containing the same concentration and internal standard with the ratios from the extracted spiked plasma and urine samples.

Clinical samples

The clinical samples analyzed in this study were obtained from patients treated for cervical cancer. IFF (3 g/m^2) was administered over 170 min by intravenous infusion. The blood samples were collected at 90, 150, 210, 240, 300, 420, 540, 750 and 1620 min after the initiation of the infusion. The plasmas were stored frozen at −20°C until analysis. The total urinary excretion was collected for 6 h periods up to 24 h after drug administration. The volumes of the samples were measured and 50-ml aliquots were stored frozen at −20°C until analysis.

Animal samples

Female Fischer rats (Charles River, Canada) weighing 150–180 g were administered a single intravenous dose of IFF (125 mg/kg) dissolved in sterile saline. Blood samples (0.3–0.5 ml) were collected from the cartotid artery at 0, 5, 15, 30, 45, 60, 90, 120, 180, 240, and 300 min.

TABLE II

INTRA-DAY VALIDATION FOR IFF AND ITS DECHLOROETHYLATED METABOLITES IN PLASMA ($n = 10$)

Theoretical concentration (μg/ml)	Measured concentration (mean ± S.D.) (μg/ml)	Accuracy (%)	C.V. (%)
(R)-IFF			
2.5	2.58 ± 0.12	103	4.6
20	20.75 ± 0.70	104	3.4
60	58.72 ± 3.22	98	5.5
(S)-IFF			
2.5	2.55 ± 0.16	102	6.3
20	20.50 ± 0.89	103	4.3
60	58.06 ± 3.43	97	5.9
(R)-2-DCE-IFF			
2.5	2.45 ± 0.09	98	3.8
30	29.10 ± 1.33	97	4.6
(S)-2-DCE-IFF			
2.5	2.30 ± 0.10	92	4.6
30	29.40 ± 1.54	98	5.2
(R)-3-DCE-IFF			
2.5	2.78 ± 0.15	111	5.4
30	30.85 ± 3.20	103	10.3
(S)-3-DCE-IFF			
2.5	2.83 ± 0.08	113	2.8
30	31.67 ± 2.01	105	6.3

The plasma collected was centrifuged and stored at −20°C until analysis.

RESULTS AND DISCUSSION

Chromatographic results

The results from the chromatography of IFF and its dechloroethylated metabolites are presented in Table I and the chromatograms obtained from blank plasma and spiked with 20 μg/ml of rac-IFF, rac-2-DCE-IFF, rac-3-DCE-IFF and rac-CP are presented in Fig. 2A and B, respectively. The enantioselective resolution of (*R*)- and (*S*)-IFF on the 2,6-Me-3-Pe-β-CD-CSP was a significant improvement over the results obtained with the Chiralsil-Val where the calculated enantioselectivity (α) and enantioselective resolution (R_{RS}) were 1.01 and 0.54, respectively [10].

The elution orders of the individual enantiomers of IFF, 2-DCE-IFF and 3-DCE-IFF were established by injecting the individual stereoisomers and unequal mixtures of the enantiomeric pairs. It is important to note that the apparent inversion in the enantiomeric elution order for 3-DCE-IFF relative to IFF and 2-DCE-IFF is a result of the Cahn–Ingold–Prelog nomenclature system and not due to the chromatographic process [2]. Thus, (*R*)-IFF is the source of (*S*)-3-DCE-IFF and (*S*)-IFF the source of (*R*)-3-DCE-IFF. Under the chromatographic conditions used in this study, the enantiomorphs of CP were not enantioselectively resolved.

The chromatographic results from the analysis of plasma and urine from a patient treated with rac-IFF (dose 3 g/m^2) and of plasma from a rat treated with rac-IFF (dose 125 mg/kg) are presented in Fig. 3A, B and C, respectively. These chromatograms show good enantioselective resolutions for all compounds and no interfering

TABLE III

INTER-DAY VALIDATION FOR IFF AND ITS DECHLOROETHYLATED METABOLITES IN PLASMA

Theoretical concentration (μg/ml)	Measured concentration (mean ± S.D.) (μg/ml)	Accuracy (%)	C.V. (%)	*n*
(R)-IFF				
2.5	2.51 ± 0.14	100	5.5	26
20	20.72 ± 1.01	104	4.9	27
60	60.84 ± 3.29	101	5.4	25
(S)-IFF				
2.5	2.58 ± 0.14	103	5.3	26
20	20.60 ± 1.10	103	5.3	27
60	60.22 ± 3.58	100	5.9	25
(R)-2-DCE-IFF				
2.5	2.34 ± 0.16	94	6.7	20
30	30.39 ± 2.26	101	7.4	20
(S)-2-DCE-IFF				
2.5	2.25 ± 0.10	90	4.7	20
30	30.32 ± 2.31	101	7.6	20
(R)-3-DCE-IFF				
2.5	2.78 ± 0.15	111	5.4	20
30	32.07 ± 2.63	106	7.4	20
(S)-3-DCE-IFF				
2.5	2.81 ± 0.11	112	3.9	20
30	31.67 ± 1.5	105	4.8	20

peaks were detected in drug-free plasma or urine, patient plasma or urine, or rat plasma for the chosen quantification range.

Comparison of human plasma and rat plasma

Identical validation studies were carried out using human and rat plasma and no significant differences were observed between the results from the two matrices. Thus, the data presented below are derived from the validation using human plasma, but are equally valid for studies involving rats.

Extraction efficiency

The recoveries for the enantiomers of IFF and the enantiomers of its dechloroethylated metabolites were studied at concentrations ranging from 2.5 μg/ml to 60 μg/ml in plasma and 2.5 μg/ml to 75 μg/ml in urine; $n = 10$ for each level. The recoveries for all compounds in plasma and urine were between 95–111% and 80–106%, respectively. However, during the validation of the urine assay, it was observed that the recoveries for the low concentrations (0.5–10 μg/ml) of 2- and 3-DCE-IFF ranged between 60–70%. The method was improved through the addition of 200 mg crystalline sodium chloride; a technique suggested by an earlier method described by Goren [13]. Using this approach, the recoveries of 2- and 3-DCE-IFF increased to 82–97% and 80–90%, respectively.

Calibrations curves

Calibrations curves for all compounds were found to be linear over the concentration range examined. The following concentrations are presented in terms of the racemic compounds. For plasma, two standard curves were used for each compound in order to improve precision; one for low concentrations [IFF (0.5–10 μg/ml; $n = 6$),

TABLE IV

INTRA-DAY VALIDATION FOR IFF AND ITS DECHLOROETHYLATED METABOLITES IN URINE ($n = 10$)

Theoretical concentration (μg/ml)	Measured concentration (mean ± S.D.) (μg/ml)	Accuracy (%)	C.V. (%)
(R)-IFF			
2.5	2.51 ± 0.13	100	5.2
30	28.55 ± 1.12	95	3.9
75	75.90 ± 2.96	101	3.9
(S)-IFF			
2.5	2.52 ± 0.11	101	4.6
30	28.92 ± 1.12	96	3.9
75	76.50 ± 2.96	102	3.9
(R)-2-DCE-IFF			
2.5	2.50 ± 0.17	100	6.8
30	30.67 ± 0.99	102	3.2
75	76.66 ± 4.48	102	5.8
(S)-2-DCE-IFF			
2.5	2.45 ± 0.14	98	5.7
30	30.55 ± 1.28	102	4.2
75	77.10 ± 4.12	103	5.3
(R)-3-DCE-IFF			
2.5	2.38 ± 0.12	95	5.3
30	30.16 ± 1.27	100	4.2
75	76.28 ± 3.76	102	4.9
(S)-3-DCE-IFF			
2.5	2.41 ± 0.14	96	5.8
30	28.84 ± 1.16	96	3.9
75	75.72 ± 3.84	101	5.1

2- and 3-DCE-IFF (1–10 μg/ml; $n = 6$)] and one for high concentrations [IFF (10–120 μg/ml; $n = 8$), 2- and 3-DCE-IFF (10–100 μg/ml; $n = 8$)]. Two standard curves were also used for the urine samples; low concentration range [IFF (1–20 μg/ml; $n = 6$), 2- and 3-DCE-IFF (1–20 μg/ml; $n = 6$)] and a high concentration range [IFF (20–200 μg/ml; $n = 8$), 2- and 3-DCE-IFF (20–200 μg/ml; $n = 8$)]. The correlation coefficients for all compounds in plasma and urine were between 0.9952 and 0.9999.

The limits of quantitation for the enantiomers of IFF and the enantiomers of 2- and 3-DCE-IFF in plasma were 250 ng/ml and 500 ng/ml respectively. In urine, the limits of quantitation for the enantiomers of IFF and the enantiomers of 2- and 3-DCE-IFF were 500 ng/ml respectively. However, the method was able to detect concentrations as low as 250 ng/ml of each enantiomer of 2- and 3-DCE-IFF in plasma and urine.

Precision and accuracy

The intra-day and inter-day precision and accuracy of this method in plasma and urine is presented in Tables II–V. In all but one case, the coefficients of variation (C.V.) were less than 8%.

Plasma and urine samples

The analytical method was applied to the simultaneous quantitation of the enantiomers of IFF and its dechloroethylated metabolites in plasma and urine from a patient undergoing

TABLE V

INTER-DAY VALIDATION FOR IFF AND ITS DECHLOROETHYLATED METABOLITES IN URINE ($n = 30$)

Theoretical concentration (μg/ml)	Measured concentration (mean ± S.D.) (μg/ml)	Accuracy (%)	C.V. (%)
(R)-IFF			
2.5	2.41 ± 0.14	96	5.8
30	27.70 ± 1.81	92	6.6
75	75.13 ± 2.80	100	3.8
(S)-IFF			
2.5	2.42 ± 0.13	97	5.4
30	28.02 ± 2.05	93	7.3
75	75.59 ± 3.00	101	3.9
(R)-2-DCE-IFF			
2.5	2.48 ± 0.16	99	6.2
30	29.09 ± 2.05	97	7.0
75	75.18 ± 3.57	100	4.7
(S)-2-DCE-IFF			
2.5	2.49 ± 0.16	99.6	6.6
30	29.20 ± 2.11	97	7.2
75	75.98 ± 3.31	101	4.4
(R)-3-DCE-IFF			
2.5	2.42 ± 0.16	97	6.9
30	28.78 ± 1.73	96	6.0
75	75.31 ± 3.50	100	4.6
(S)-3-DCE-IFF			
2.5	2.42 ± 0.19	97	7.8
30	28.10 ± 1.52	96	5.4
75	74.71 ± 3.51	99.6	4.7

treatment with rac-IFF and in plasma from a rat which had also received rac-IFF. Representative serum concentration–time curves are presented in Figs. 4 and 5. When analyzing this data, the reader is again reminded that (*S*)-IFF produces (*S*)-2-DCE-IFF and (*R*)-3-DCE-IFF while (*R*)-IFF produces (*R*)-2-DCE-IFF and (*S*)-3-DCE-IFF.

In the human, the plasma concentrations of (*R*)-IFF were higher than the corresponding concentrations of (*S*)-IFF at all time points; the ratio of (*R*)-IFF to (*S*)-IFF was 1.03:1 at the end of infusion and increased to 2.35:1 at 16 h, Fig. 4A. These results are in agreement with previously reported studies of the plasma clearance of (*R*)- and (*S*)-IFF in humans [2,7]. The same pattern was not observed in rat plasma where the (*R*)-IFF to (*S*)-IFF ratios remained close to unity during the sampling period, Fig. 5A.

The principal circulating N-dechloroethylated metabolite in both human and rat plasma was (*R*)-3-DCE-IFF, a metabolite of (*S*)-IFF, Figs. 4B and 5B. In human plasma, the relative concentrations of the remaining metabolites were (*S*)-3-DCE-IFF > (*S*)-2-DCE-IFF > (*R*)-2-DCE-IFF, Fig. 4B. (*R*)-2-DCE-IFF was detected in some rats after 3 h at a concentration below 500 ng/ml. The relative concentrations of the remaining two metabolites were (*S*)-3-DCE-IFF > (*S*)-2-DCE-IFF.

The percentage cumulative excretions of the enantiomers of IFF and 2- and 3-DCE-IFF relative to administered dose from one patient are presented in Fig. 6. The percentage cumula-

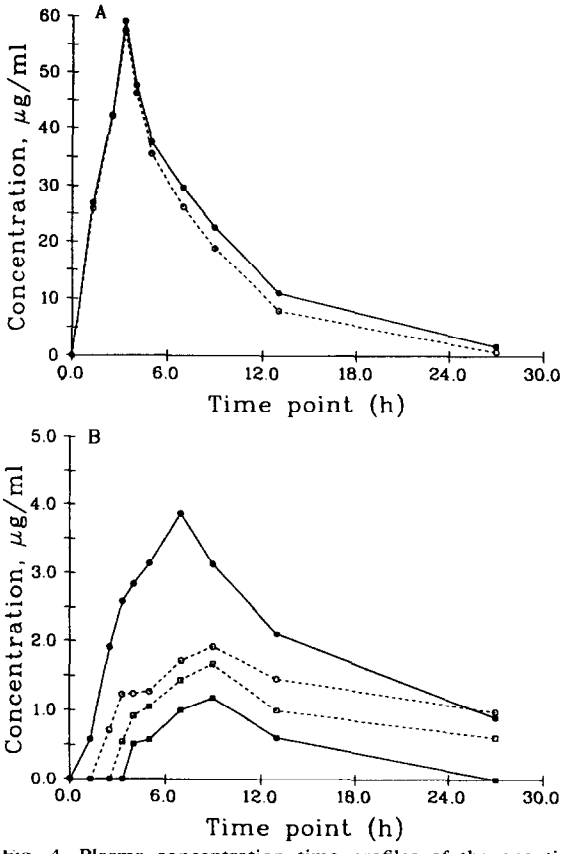

Fig. 4. Plasma concentration–time profiles of the enantiomers of IFF (A) and its dechloroethylated metabolites (B) from a patient treated with 3 g/m^2 rac-IFF infused i.v. over 170 min. Where: A: (●) (*R*)-IFF, (○) (*S*)-IFF; B: (●) (*R*)-3-DCE-IFF, (○) (*S*)-3-DCE-IFF, (■) (*R*)-2-DCE-IFF, (□) (*S*)-2-DCE-IFF.

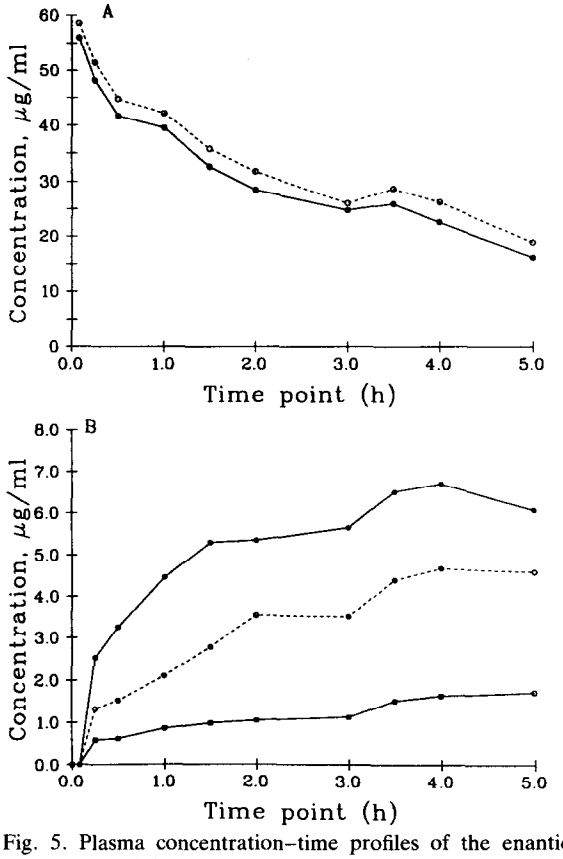

Fig. 5. Plasma concentration–time profiles of the enantiomers of IFF (A) and its dechloroethylated metabolites (B) following intravenous administration of 125 mg/kg of rac-IFF in the rat. Where: A: (●) (*R*)-IFF, (○) (*S*)-IFF; B: (●) (*R*)-3-DCE-IFF, (○) (*S*)-3-DCE-IFF, (□) (*S*)-2-DCE-IFF.

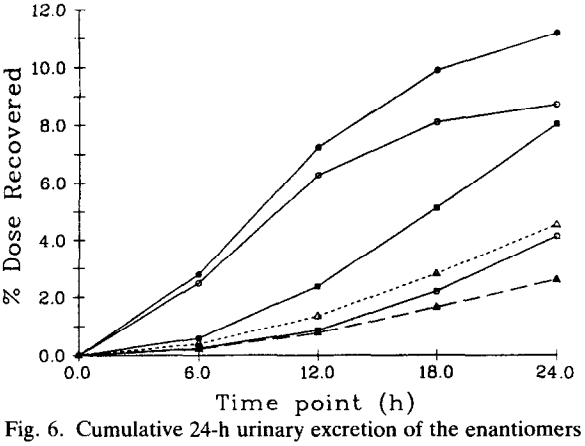

Fig. 6. Cumulative 24-h urinary excretion of the enantiomers of IFF and 2- and 3-DCE-IFF expressed as percentage of administered dose from a patient treated with 3 g/m^2 rac-IFF infused i.v. over 170 min. Where: A: (●) (*R*)-IFF, (○) (*S*)-IFF; (■) (*R*)-3-DCE-IFF, (□) (*S*)-3-DCE-IFF, (▲) (*R*)-2-DCE-IFF, (△) (*S*)-2-DCE-IFF.

tive 24-h urine excretion was 20% for IFF, 7.2% for 2-DCE-IFF and 12.21% for 3-DCE-IFF. These results are consistent with the data reported by Boss et al. [2] for twelve children [IFF (14–34%), 2-DCE-IFF (2–8%), 3-DCE-IFF (9–29%)], and by Goren [13] for five patients [IFF (11–30%), 2-DCE-IFF (3–10%), 3-DCE-IFF (11–21%)].

CONCLUSION

The GC–MS method described in this paper is sensitive, reproducible, precise, and uses a very stable 2,6-di-O-methyl-3-O-pentyl-β-cyclodextrin CSP. The method was found to be applicable to

the study of the pharmacokinetics of IFF and its dechloroethylated metabolites in biological samples and is the first reported assay capable of measuring the enantiomeric concentrations of 2- and 3-DCE-IFF in human and animal plasma.

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