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Short Communication

Determination of intoplicine, a new antitumour drug, in human faeces by normal-phase high-performance liquid chromatography with fluorescence detection*

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Introduction

Intoplicine (RP60475F, NSC D645008, 11-(3-dimethylaminopropylamino)-3-hydroxy-8-methyl-7H-benzo[e]pyrido[4,3-b]-indole, dimethanesulphonate, Fig. 1) [1], a novel, potential antitumour drug, is now undergoing Phase-1 clinical evaluation in the US and Europe. To study the pharmacokinetics of a new drug is one of the objectives of a Phase-1 study. A sensitive and selective high-performance liquid chromatographic (HPLC) method for the analysis of intoplicine in both plasma and whole blood samples has been described [2]. Investigations with radiolabelled intoplicine, being administered to animals, demonstrated that the radioactivity is mainly recovered in faeces [personal communication, Rhône-Poulenc Rorer, Antony, France]. This may indicate that faecal excretion is also a major route for the elimination of the drug in humans. However, there are no data in the literature on this topic. For the determination of intoplicine in human faeces, adaptation and validation of our earlier reported HPLC assay of intoplicine [2] was required due to interferences in the extracts. In this paper a selective and sensitive assay for the determination of intoplicine in human faeces is described. The reported assay was validated and pharmacokinetic results of a patient who received 1080 mg of intoplicine (640 mg m^{-2}) as a 24 h infusion are given.

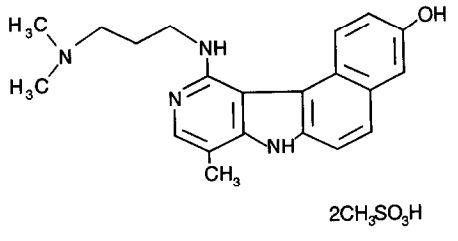

Figure 1
Structure of intoplicine dimethanesulphonate.

Experimental

Chemicals

Intoplicine (as dimethanesulphonate) originated from Rhône-Poulenc Rorer Company (Antony Cedex, France). Dichloromethane was obtained from Mallinckrodt (Paris, KY, USA), ammonia (25% w/v), sodium hydroxide, hydrochloric acid (37% w/v), disodium tetraborate (all analytical grade) and 2-propanol Lichrosolv® originated from Merck (Darmstadt, Germany). Distilled water produced in the pharmacy department of the hospital was used throughout.

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Calibration samples

A stock solution of intoplicine (1 mg ml^{-1}) was prepared in water and, if stored at −30°C, was stable for at least 2 months. This stock solution was diluted with distilled water to give standard solutions with concentrations of 10000, 1000 and 100 ng ml^{-1}. Calibration samples were prepared from spiked drug-free faeces suspensions (100 μg faeces ml^{-1} in water); the drug concentration range was 10–10000 ng g^{-1} faeces.

Patient samples

A 63-y old female patient received 1080 mg (640 mg m^{-2}) of intoplicine as an intravenous infusion over 24 h. Faeces was obtained before, during and over a period of 96 h after infusion. After every motion the stools were collected and stored at −30°C.

Sample pretreatment

The human faeces samples were thawed and homogenized in distilled water (10:90, w/w) by means of an Ystral homogenizer (Salm & Kipp, Breukelen, The Netherlands). To a volume of homogenate (1000 μl), 0.1 N hydrochloric acid (50 μl) and *n*-hexane (10.00 ml) were added in a polypropylene tube (30 ml). The tube was shaken for 10 min and, next, centrifuged for 5 min at 1600***g***. The organic layer was discarded and a 6% borate buffer (2.00 ml, prepared by dissolving 6.0 g of disodiumtetraborate in warm (50°C) distilled water, pH 9) and dichloromethane (10.00 ml) were added. This mixture was shaken for 10 min and then centrifuged for 5 min at 1600***g***. Next, 8.00 ml of the organic layer was transferred to a polypropylene tube (10 ml) and evaporated to dryness under nitrogen (40°C). The residue was redissolved in the mobile phase (1000 μl) and an aliquot (10 μl) was injected into the HPLC system (if necessary, the samples were diluted 10 or 100 times with the mobile phase).

Chromatography

The HPLC system consisted of a solvent delivery system type 510 (Waters, Milford, MA, USA), a LS 40 fluorescence detector (Perkin–Elmer, Norwalk, CT, USA), a SP 8880 automatic sample injection device and a SP 4270 integrator (Spectra Physics, San Jose, CA, USA). The analytical glass column (100 × 3.0 mm internal diameter) was packed with ChromSpher® 5 μm silica material (Chrompak BV, Bergen op Zoom, The Netherlands) and was protected by a Chromspher guard column (10 × 2.1 mm internal diameter; packed with 5 μm Silica, Chrompack). Chromatographic analyses were performed at ambient temperature with a mobile phase composed of dichloromethane–2-propanol–ammonia (10%, v/v) (40:40:1, v/v/v). Prior to use, dichloromethane and 2-propanol were filtered through a 0.22 μm filter. The fluorescence was monitored with excitation and emission wavelengths set at 375 and 425 nm, respectively. The flow rate was maintained at 0.6 ml min^{-1}.

Validation

The absolute recovery of the extraction procedure has been calculated by dividing the slope of the calibration line in the biological matrix by the slope of the calibration line in the mobile phase (untreated standards). The accuracy and precision of the method were determined by replicate analysis of known concentrations at four levels equally divided over the calibration curve. Peak areas were used for quantitative computations. Calibration curves were calculated by 'weighted' ($1/x$) least squares linear regression analysis using a commercial software package (NCSS, Kaysville, UT, USA). The detection limit is the concentration of the drug where the fluorescence response is equal to three times the average noise signal. The lower limit of quantitation is defined as the concentration of the lowest standard in the analytical run which is quantitated with a definite level of certainty (precision <20%).

Results and Discussion

Method development

For the quantification of intoplicine in human faeces the recovery of each isolation and purification step has been determined and optimized. To remove lipids, and to promote the homogeneity of the specimen, faeces samples were extracted first with *n*-hexane at pH 3. No significant loss of intoplicine occurred. Next, the drug is extracted from the faeces samples with dichloromethane at pH 9 and the organic extract is evaporated to dryness (nitrogen, 40°C). A representative HPLC chromatogram of the analysis of patient faeces is shown in Fig. 2. Analysis of five different blank faeces samples, did not show any interferences in the chromatograms.

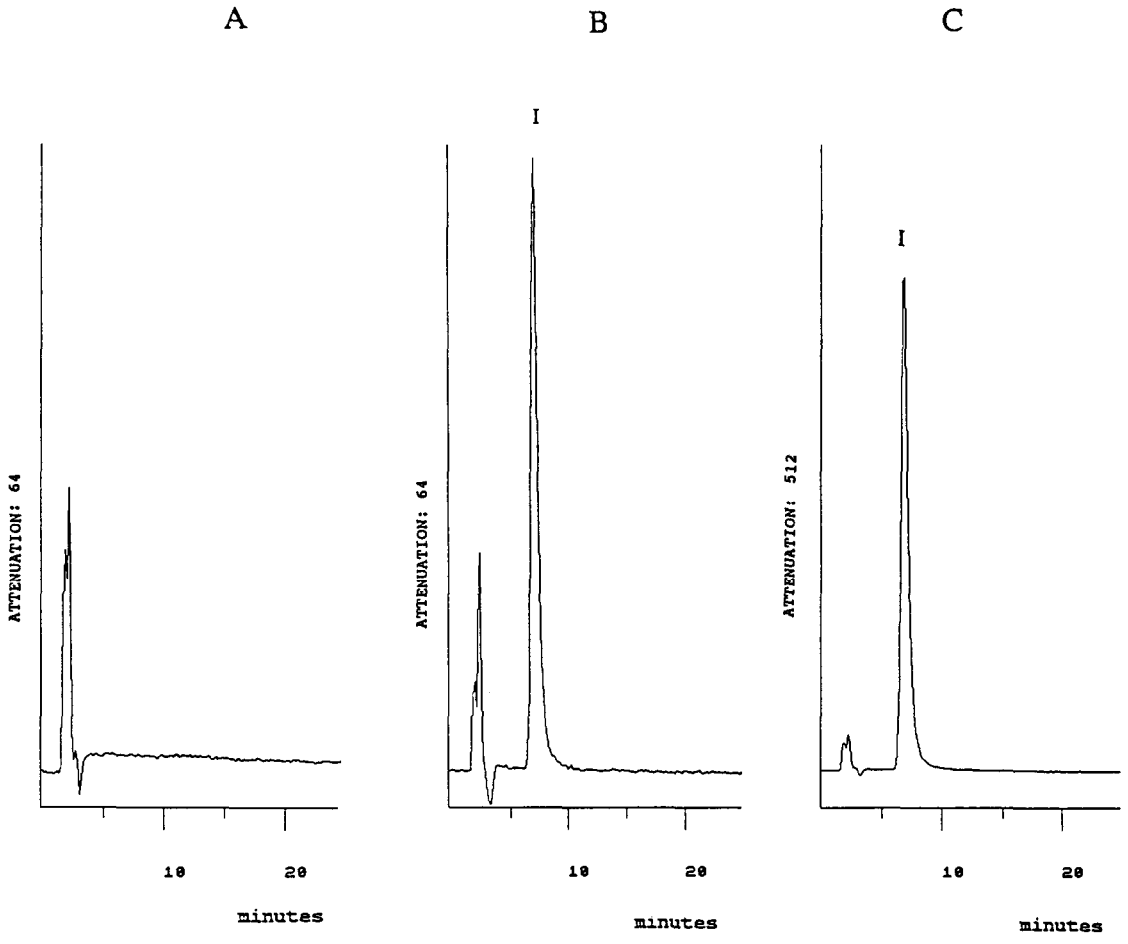

Figure 2
HPLC of blank faeces (A), a calibration sample (B) (concentration of intoplicine = 500 ng g^{-1}) and a faeces sample (C) (concentration of intoplicine = 6.7 μg g^{-1}) of a patient receiving 1080 mg as a 24-h intravenous infusion. The retention time of intoplicine (I) is 6.5 min.

Table 1
Accuracy and precision for the bio-analysis of intoplicine in faeces

Theoretical conc. (ng ml^{-1})	Measured conc. (ng ml^{-1})	Accuracy (%)	Precision* Between-day (%)	Precision* Within-day (%)	n†
50.0	45.6	91.1	2.1	7.1	5
500	504	100.7	1.0	2.7	5
5000	5803	116.0	1.4	2.9	5
10000	9853	98.5	2.0	1.6	5

*Between-day precision was performed on three subsequent occasions (once a week).
†n = number of replicates.

Validation

The absolute recovery of intoplicine was 60.1 ± 4.9% (n = 3) over the whole concentration range. The limit of detection was 10 ng g^{-1} using 1000 μl of the faeces suspension and the lower limit of quantitation was 50 ng g^{-1}. Accuracy and precision parameters of the assay have been tabulated (Table 1) and are acceptable, not requiring an internal standard. The calibration lines in faeces were linear ($r > 0.996$, $P < 0.0001$) over the concentration range of interest (50 ng g^{-1}–10 μg g^{-1}). The excretion profile of intoplicine in faeces in one individual is shown in Fig. 3.

The sensitivity of the assay was sufficient to determine the concentration in the samples

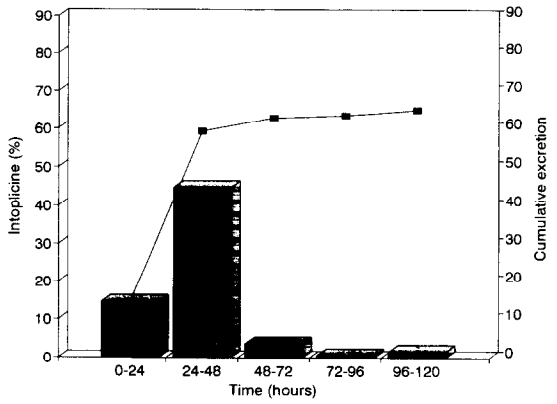

Figure 3
Excretion of unchanged intoplicine, given as percentage of the administered dose, in faeces (excretion per interval in bars; cumulative excretion as —■—).

collected 96–120 h after the start of the infusion. Possible metabolic products of intoplicine were not detected, even overnight (12 h) HPLC analysis did not show any other compounds in the chromatograms.

Conclusions

In conclusion, the reported assay is suitable for the determination of intoplicine in faeces. The pharmacokinetic results of one patient showed that the unchanged drug was excreted in faeces up to 65% of the administered dose within 5 days after the infusion with most of the intoplicine being excreted during the 24–48 h interval (0–24 h after the infusion). This result indicates that a major part of the administered dose is excreted, unchanged, in faeces.

References

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