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Short Communication

Quantitation of 6-(pyridin-3-yl)quinolin-2(1H)-one, a cardiac stimulant, and its N-oxide metabolite in dog plasma by high-performance liquid chromatography

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ABSTRACT

A method is described for the determination of UK-57,400 (I), a 6-substituted quinoline cardiotonic, and its pyridyl-N-oxide in dog plasma. The analytes are selectively retained from plasma (1 ml) on a solid-phase extraction column and eluted with 1 ml of methanol. After evaporation to dryness, the residue is reconstituted in 100 μl of the mobile phase. Chromatography is carried out on a Spherisorb 5 μm phenyl high-performance liquid chromatography column, with ultraviolet detection. Calibration curves are linear for concentrations from 10 to 100 ng ml^{-1}. For I, the coefficients of variation at highest and lowest concentrations are 1 and 14%, respectively, while the corresponding figures for the pyridyl-N-oxide metabolite are 4 and 10%, respectively. Sample recovery from extraction is greater than 90%. The limit of detection is 4 ng ml^{-1} for both analytes.

INTRODUCTION

6-(Pyridin-3-yl)quinolin-2(1H)-one (UK-57,400, I, Fig. 1) has been reported to have positive inotropic and vasodilatory properties [1–3]. It increases myocardial contractile force with little tachycardia and therefore it was considered to be of potential value in the treatment of congestive heart failure (CHF). Compound I was a prototype of the series of novel inotropes from which dimethylimidazolequinolin-2(1H)-one (UK-61,260) was progressed to clinical evaluation [4]. The principal metabolite of I is the pyridyl-N-oxide (UK-59,572, II, Fig. 1) [5]. To facilitate a study of the pharmacokinetics of I, we have developed a sensitive and selective method for the determination of the drug and its N-oxide metabolite in dog plasma using high-performance liquid chromatography (HPLC) with ultraviolet detection.

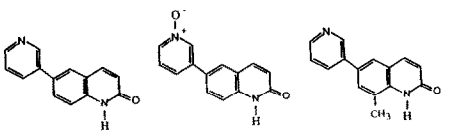

Fig. 1. Structures of I (UK-57,400), II (UK-59,572) and III (the internal standard, UK-59,669).

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EXPERIMENTAL

Chemicals and reagents

Compounds I, II and III (internal standard, UK-59,669) were synthesised at Pfizer Central Research (Sandwich, UK). Ammonium dihydrogenphosphate and N,N,N′,N′-tetramethylethylenediamine (TEMED) were purchased from Fluka (Glossop, UK). HPLC-grade methanol was obtained from Fisons (Loughborough, UK). All the other reagents of HPLC or analytical grade were purchased from commercial sources and used without further purification. Water was deionised, doubled distilled, and the prepared mobile phase was filtered through a Nylon 66, 0.2 μm Sartorius filter (Sartorius, G.-B. Belmont, UK).

Chromatograpy

The HPLC system consisted of a Water Assoc. (Milford, MA, USA) Model A-6000 solvent delivery system, Spectra-Physics autosampler (Santa Clara, CA, USA) and Shimadzu UV spectrophotometer (Duisburg, Germany). The separation system was a 12.5 cm × 0.46 cm I.D. stainless-steel Spherisorb 5 μm phenyl column (Hichrom, Reading, UK). The signal output from the detector was quantitated on a Spectra-Physics integrator. The mobile phase consisted of 80% ammonium dihydrogenphosphate (50 m*M*) containing 1% (v/v) (0.067 *M*) TEMED and 20% methanol, adjusted to pH 5 with glacial acetic acid (17.4 *M*), which was run at a flow-rate of 1 ml min^{-1}. The column eluent was monitored at 254 nm. All analyses were performed at room temperature.

Reference standards

Standard solutions of concentrations of 10 μg ml^{-1} I, II and III were made up in methanol. These stock solutions of the analyses and internal standard were stable at 4°C for six months.

Preparation of samples

A close structural analogue of I, III, was used as an internal standard (Fig. 1). A 20-μl volume of a 10 μg ml^{-1} stock solution was added to a series of Pyrex glass borosilicate disposable tubes, 13 mm × 10 cm. The calibration curves were constructed by adding the following concentrations: 100, 60, 40, 20 and 10 ng of the analytes to tubes containing 1 ml of control dog plasma.

Extraction procedure

After the tubes were vortex-mixed, a CH Bond-Elut extraction cartridge (100 mg) (Anachem, Luton, UK) was conditioned with 1 ml of methanol, followed by 1 ml of distilled water. The samples (1 ml) were then transferred from the glass culture tubes to the pre-conditioned Bond-Elut cartridges *via* borosilicate glass pipettes. The samples were adsorbed onto the column by applying mild vacuum (VacElut, Anachem). The cartridges were washed with 1 ml of a mixture of methanol–water (20:80). The analytes and internal standard were eluted with 1 ml of methanol. The eluate was collected and dried at 37°C in a water bath, under a stream of nitrogen. The residue was reconstituted in 100 μl of the mobile phase and vortex-mixed for 10 s. Aliquots of 60 μl were injected directly onto the liquid chromatograph.

Quantitation, analytical precision and recovery

Quantitation was by comparison of the peak-area ratio of the analytes to internal standard using the standard calibration curves.

Recovery of I, its N-oxide metabolite (II) and the internal standard (III) was estimated by comparing the peak area obtained from a spiked plasma sample with that of a control extract subsequently spiked with an identical amount of each reference compound.

RESULTS AND DISCUSSION

During the course of the method development, liquid–liquid extraction was investigated but yielded poor results for the N-oxide (II), owing to its low extraction efficiency. The wide applicability of bonded-phase silica in separation science indicated a potential alternative. Indeed, a higher degree of extraction efficiency was achieved for the N-oxide when the solid-phase procedure was employed. Moreover, the technique was less time-consuming.

Typical chromatograms obtained from plasma

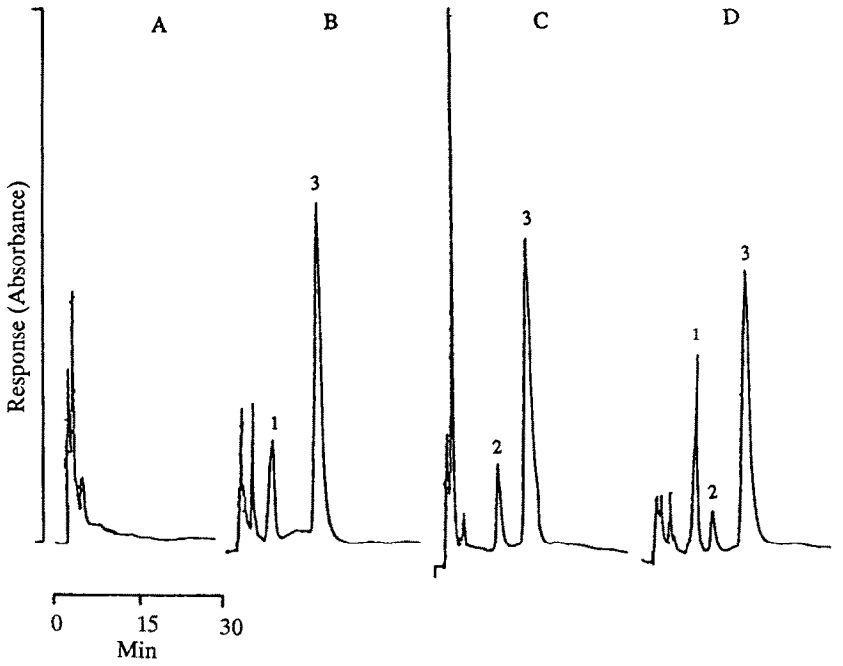

Fig. 2. Chromatograms obtained from extractions of (A) blank dog plasma, (B) plasma spiked with II (20 ng ml^{-1}) and III (internal standard), (C) plasma spiked with I (20 ng ml^{-1}) and III, and (D) plasma from a dog dosed with an intravenous dose of 0.5 mg/kg I. Peaks (retention times in parentheses): 1 = II (8 min); 2 = I (11 min); 3 = III (16 min).

standards and dog plasma sample are illustrated in Fig. 2. Under the chromatographic conditions employed, I, II and the internal standard III were well resolved from endogenous peaks. The mobile phase at pH 5 without TEMED produced non-symmetrical peaks; addition of TEMED, an aliphatic amine, effectively suppressed peak tailing, probably by masking the residual silanol groups. The presence of TEMED was observed to cause faster elution of I and III but did not affect the elution of II which was mainly controlled by the composition of the organic component of the mobile phase, possibly due to the reduced basicity resulting from N–O bond formation.

TABLE I

SUMMARY OF ACCURACY AND PRECISION DATA OF ASSAY FOR I AND II

Concentration added (ng ml^{-1})	Concentration found (mean ± S.D., $n=6$) (ng ml^{-1})	C.V. (%)
Compound I		
100	100.0 ± 0.7	1
50	50.5 ± 1.1	2
10	8.1 ± 1.2	14
Compound II		
100	94.4 ± 3.6	4
50	48.7 ± 2.3	5
10	10.3 ± 1.0	10

Recoveries of I, II and the internal standard were 95, 92 and 91%, respectively. Linear regression analysis of standard curves, over the range of 10–100 ng ml^{-1}, on five consecutive assays (done on different days), showed correlation coefficients of 0.9990 and 0.9986 for II and I, respectively. The coefficients of variation (C.V.) of the slopes were 5.6% for I and 2.8% for II which represent the variability in the slopes of standard curves made from extracted standard samples. The assay precision data are summarised in Table I. A little variation in the assay of II with the C.V. below 10% was observed while the corresponding value of I was slightly greater at low concentrations tested. The detection limits were 4 ng ml^{-1} for both I and II with a signal-to-noise ratio of 4:1.

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