Journal of Chromatography, 182 (1980) 459—464
Biomedical Applications

CHROMBIO. 561

Note

High-performance liquid chromatographic determination of chloramphenicol and its monosuccinate ester in plasma

KAREN B. OSEEKEY, KIM L. ROWSE and HARRY B. KOSTENBAUDER

The University of Kentucky, College of Pharmacy, Washington and Gladstone Sts., Lexington, KY 40506 (U.S.A.)

(Received January 3rd, 1980)

When used parenterally, chloramphenical is administered as a water-soluble salt of the monosuccinate ester of chloramphenicol. Because plasma samples obtained shortly after intravenous administration may contain both chloramphenicol and the monosuccinate pro-drug, and because urine has been reported to contain up to 30% of the chloramphenicol dose in the form of the unhydrolyzed monosuccinate ester [1], it is necessary that analytical methods be capable of distinguishing between these species. Furthermore, it is necessary that specimens be processed in a manner which avoids in vitro conversion of the monosuccinate pro-drug to chloramphenicol.

Several high-performance liquid chromatographic (HPLC) assays for chloramphenicol in biological fluids have been published [2—6]. One procedure [4], using drug extraction from biological fluids adjusted to pH 10.4, would be expected to effectively separate chloramphenicol and hemisuccinate ester in the extraction step, although there was no consideration of potential for error due to hydrolysis of the monoester pro-drug to chloramphenicol in pH 10.4 buffer. The present communication addresses the above question and also presents methodology for HPLC quantitation of both chloramphenicol and its monosuccinate ester pro-drug in plasma.

EXPERIMENTAL

Apparatus

The high-performance liquid chromatographic system consisted of a Milton Roy Minipump (Laboratory Data Control, Riviera Beach, FL, U.S.A.) equipped with a variable-wavelength spectrophotometric detector, Vari-chrom (Varian, Palo Alto, CA, U.S.A.) and a Houston Instrument OmniScribe recorder (Austin, TX, U.S.A.). A Rheodyne loop injector (Berkeley, CA, U.S.A.) with a 100-μl

sampling loop was used to introduce the samples onto a LiChrosorb C_8 reversed-phase column, 10 μm (Brownlee Labs., Santa Clara, CA, U.S.A.). During the development of the calibration curves, a Waters Assoc. WISP 710A automatic injector (Milford, MA, U.S.A.) was used.

Operation conditions

The variable wavelength UV-VIS detector was set at 280 nm. The sensitivity of the detector was 0.05 a.u./10 mV output. The flow-rate was held constant at 2.5 ml/min. The column pressure was between 2000 and 3000 p.s.i.

Mobile phase

The mobile phase, methanol—0.05% aqueous phosphoric acid (4:6), was prepared by direct admixture and degassed by water pump aspiration for 5 min.

Routine sample preparation

To 0.4 ml plasma in 16 × 125 mm culture tubes were added 50 μl ethylparaben (Aldrich, Milwaukee, WI, U.S.A.) (20 mg%) as internal standard, 1 ml of a 0.1 *M* citric acid—0.2 *M* Na_2HPO_4 (803:197) pH 3.0 aqueous buffer, and 5 ml anhydrous diethyl ether. The contents were mixed for 30 sec via gentle tube inversion to prevent gel formation and were then centrifuged for 3 min. The organic layer was transferred and evaporated to dryness via a gentle air stream at room temperature. The samples were then reconstituted with 100 μl of mobile phase, and 20-μl aliquots were chromatographed.

Pooled human plasma samples supplemented with chloramphenicol concentrations up to 40 μg/ml were also analyzed to establish standard calibration curves on a daily basis.

Standards for assay validation

Standard curves were developed for chloramphenicol (Aldrich) and the 3-monosuccinate ester of chloramphenicol. Chloramphenicol-3-monosuccinate was isolated by acidifying a solution of chloramphenicol sodium succinate for injection (Parke, Davis and Co., Detroit, MI, U.S.A.). The precipitate was collected and recrystallized from a water—methanol mixture.

Samples containing from 2.5 to 40.0 μg/ml chloramphenicol or chloramphenicol-3-monosuccinate and 20 μg/ml ethylparaben (internal standard) were prepared in pooled human plasma. Extractions of six samples of each of the five concentrations were done prior to chromatography for both the chloramphenicol and chloramphenicol-3-monosuccinate samples.

Direct HPLC injections of aqueous samples of the same concentrations of chloramphenicol and its 3-monosuccinate ester were also chromatographed to assess the plasma extraction recovery.

Stability of chloramphenicol-3-monosuccinate

Aliquots of 20 μl of a solution containing 1 ml chloramphenicol-3-monosuccinate aqueous (1 mg/ml), 1 ml ethylparaben aqueous (25 mg%), and 8 ml of citric acid—phosphate pH 3.0 buffer were chromatographed at 20-min intervals for seven injections.

Similarly, direct 20-μl injections of a solution buffered with 8 ml of 0.8 *M*

tris(hydroxymethyl) aminomethane (Tris) pH 10.4 buffer were also chromatographed.

RESULTS AND DISCUSSION

Typical chromatograms for chloramphenicol and chloramphenicol-3-monosuccinate aqueous standards are shown in Fig. 1. Under these chromatographic

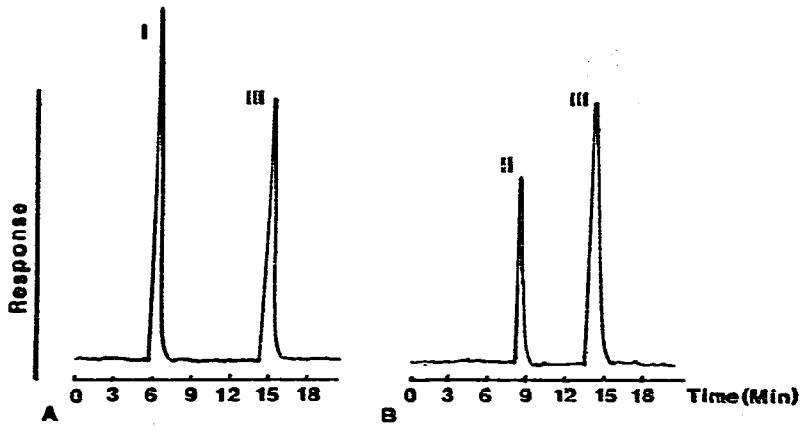

Fig. 1. Representative chromatograms obtained from direct HPLC 20-μl injections of (A) 10 μg/ml aqueous chloramphenicol; (B) 10 μg/ml aqueous chloramphenicol-3-monosuccinate. Peaks: I, chloramphenicol; II, chloramphenicol-3-monosuccinate; III, ethylparaben (internal standard).

conditions, chloramphenicol, its 3-monosuccinate ester, and ethylparaben (internal standard) have retention times of 6, 9, and 15 min, respectively. Retention times were unchanged with the samples of chloramphenicol and chloramphenicol-3-monosuccinate extracted from plasma.

The major metabolites, the 3-glucuronide and deacylated form of chloramphenicol [7] are more polar than the parent drug. Under the reversed-phase separation conditions, they would be eluted early from the column and not interfere with the drug peak [2,4].

The precision of this method was determined by assaying samples of known concentrations of chloramphenicol and its 3-monosuccinate ester. The results of this study, which involved extraction of the drug from plasma, are given in Table I. Peak height ratios (peak height drug/peak height internal standard) were used for quantitation of drug concentration. From comparison of chloramphenicol and chloramphenicol-3-monosuccinate peak heights obtained from direct injection of aqueous solutions and from samples carried through the assay procedure, extraction efficiency was estimated as 51% (coefficient of variation 10%, $n = 5$) for chloramphenicol and 45% (coefficient of variation 8%, $n = 5$) for the 3-monosuccinate ester.

It was desired to demonstrate that citric acid—phosphate pH 3.0 buffer used in the sample preparation did not cause hydrolysis of chloramphenicol-3-monosuccinate and at the same time determine the stability of the ester to 0.8 *M* Tris pH 10.4 buffer. A constant peak height ratio obtained over a 2-h period

TABLE I

PRECISION OF EXTRACTION PROCEDURE AND ANALYSIS OF CHLORAMPHENICOL AND CHLORAMPHENICOL-3-MONOSUCCINATE FROM PLASMA

$n = 6$.

Concentration (μg/ml)	Peak height ratio (mean)	Standard deviation	Coefficient of variation (%)
Chloramphenicol			
2.5	0.304	0.013	4.1
5.0	0.601	0.032	5.3
10.0	1.237	0.021	1.7
25.0	3.218	0.081	2.5
40.0	5.241	0.227	4.3
Chloramphenicol-3-monosuccinate			
2.5	0.177	0.019	10.5
5.0	0.344	0.018	5.2
10.0	0.655	0.006	0.9
25.0	1.718	0.054	3.1
40.0	2.881	0.119	4.1

for chloramphenicol-3-monosuccinate buffered with citric acid—phosphate pH 3.0 buffer substantiated the stability of the drug in this system (Fig. 2).

In the 0.8 *M* Tris pH 10.4 buffer system, hydrolysis of the ester to chloramphenicol occurred slowly; a third peak observed with a retention time of 7.5 min was clearly related to drug concentration (Fig. 3). Sandmann and coworkers [8,9] found that the anion of the 3-monosuccinate ester in aqueous solution at near neutral pH formed an equilibrium mixture with a form which Sandmann identified as a rearranged cyclic hemi-"ortho"-succinate ester of the

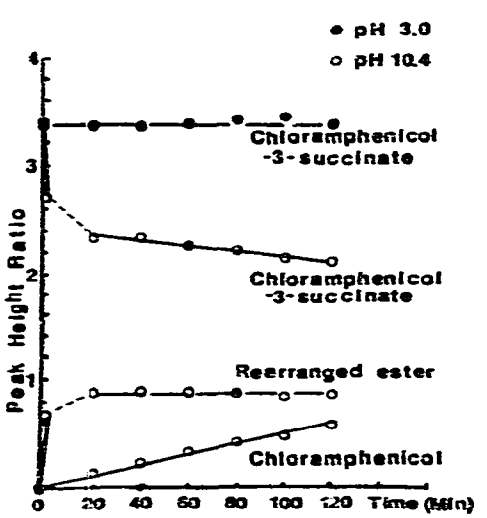

Fig. 2. Peak height ratio (drug species/internal standard) vs. time of direct HPLC injections following addition of citric acid—phosphate pH 3.0 buffer or 0.8 *M* Tris pH 10.4 buffer to aqueous chloramphenicol-3-monosuccinate.

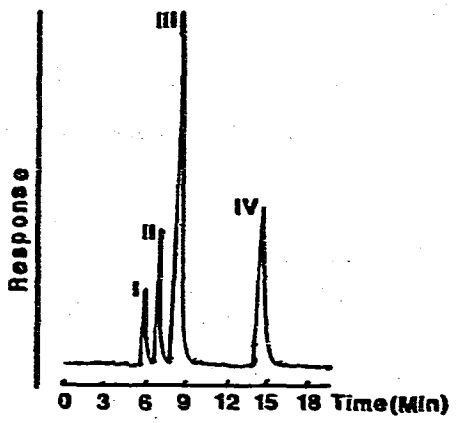

Fig. 3. Chromatogram obtained from direct HPLC injection of aqueous chloramphenicol-3-monosuccinate to which 0.8 *M* Tris pH 10.4 buffer had been added 100 min prior to injection. Peaks: I, chloramphenicol; II, rearranged ester; III, chloramphenicol-3-monosuccinate; IV, ethylparaben (internal standard).

linear 3-monosuccinate ester of chloramphenicol.

The HPLC results of the alkaline buffered solution of chloramphenicol-3-monosuccinate over a 2-h period are represented in Fig. 2. This clearly demonstrates a dramatic decrease in the concentration of chloramphenicol-3-monosuccinate when the solution is buffered at pH 10.4. A 20% rearrangement of this ester occurred immediately; after 20 min, rearrangement to the extent of 30% was observed. Hydrolysis of the esters to chloramphenicol occurred only to the extent of 2% within 20 min. Assuming that the rearranged ester has an absorptivity similar to that for the linear ester, it may be deduced from the peak height ratios for the esters that only 5% of the esters hydrolyzed to chloramphenicol after 1 h exposure to 0.8 *M* Tris pH 10.4 buffer. Confirmation of the hydrolysis was made by calculation using a calibration curve for chloramphenicol.

When chloramphenicol-3-monosuccinate was left in contact with pH 10.4 buffer for 144 h, the rearranged ester followed a first-order decline which paralleled the disappearance of the peak corresponding to the linear ester after approximately 24 h.

In conclusion, this methodology can be used to quantitate chloramphenicol and the monosuccinate ester(s) of chloramphenicol in plasma. Chloramphenicol-3-monosuccinate is stable to hydrolysis for a minimum of 2 h when buffered with a citric acid—phosphate pH 3.0 buffer. No internal rearrangement of the linear ester occurs with this buffer. Although the use of 0.8 *M* Tris pH 10.4 buffer causes rapid rearrangement of the linear 3-monosuccinate ester of chloramphenicol, hydrolysis of ester to chloramphenicol occurs slowly. Consequently, unless this buffer remains in contact with the ester(s) for prolonged periods, it is unlikely that any ester present in the plasma will be erroneously reported as chloramphenicol.

REFERENCES

1 A.J. Glazko, W.A. Dill, A.W. Kinkel, J.R. Goulet, W.J. Holloway and R.A. Buchanan, Clin. Pharmacol. Ther., 21 (1977) 104.
2 J.R. Koup, B. Brodsky, A. Lau and T.R. Beam, Jr., Antimicrob. Ag. Chemother., 14 (1978) 439.
3 G.W. Peng, M.A.F. Gadalla and W.L. Chiou, J. Pharm. Sci., 67 (1978) 1036.
4 R.L. Thies and L.J. Fischer, Clin. Chem., 24 (1978) 778.
5 J. Crechiolo and R.E. Hill, J. Chromatogr., 162 (1979) 460.
6 J.M. Wal, J.C. Peleran and G. Bories, J. Chromatogr., 145 (1978) 502.
7 A. Glazko, in G.L. Hobby (Editor), Proc. 6th Intersci. Conf. Antimicrob. Ag. Chemother., Philadelphia, PA, October 26—28, 1966, American Society for Microbiology, Ann. Arbor, MI, 1967, p. 655.
8 B. Sandmann, D. Szulczewski, J. Windheuser and T. Higuchi, J. Pharm. Sci., 59 (1970) 427.
9 B.J. Sandmann, Ph.D. dissertation, University of Wisconsin, Madison, WI, 1969.