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Journal of Chromatography B, 755 (2001) 143–149

JOURNAL OF CHROMATOGRAPHY B

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Sensitive and specific determination of clindamycin in human serum and bone tissue applying liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry

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Received 25 July 2000; received in revised form 29 December 2000; accepted 17 January 2001

Abstract

A method for the quantification of clindamycin in human serum and in human bone tissue samples applying high-performance liquid chromatography with atmospheric pressure chemical ionization–mass spectrometry (APCI–MS) is presented. Lincomycin is used as the internal standard. Serum samples are prepared only by protein precipitation with acetonitrile. Bone tissue samples have to be crushed and homogenized in extraction buffer prior to analysis. The chromatographic separation is achieved on an RP-18 stationary phase with 0.02% trifluoroacetic acid in water 60%/acetonitrile 40% v/v as mobile phase. The limits of quantification are 0.1 μg/ml for serum samples and 0.1 μg/g for bone tissue samples. The coefficients of variation for the assays are 4.48 and 8.41% at the limit of quantification for serum and bone tissue samples, respectively. Bone tissue samples as small as 50 mg can be used.

Keywords: Clindamycin

1. Introduction

Clindamycin is a lincosamide antibiotic. It is effective in the treatment of most infections involving anaerobes and gram-positive bacteria, but it is generally inactive against enterococcus and gram-negative aerobes. One of its therapeutic uses is the prophylaxis and the treatment of bone infections in the course of dental operations [1]. Of special interest in this context are the concentration values of clindamycin in the concerned regions of the bone tissue and the concentration quotient of bone tissue and serum.

Procedures for the determination of clindamycin in human serum utilizing HPLC separation with UV-detection are described in the literature [2–4]. They are either not very sensitive or time-consuming and labor-intensive. A recent publication described an HPLC–electrospray (ESI) tandem mass spectrometry assay for clindamycin from human plasma [5]. Because of the selective detector, the samples need only very little preparation. But the well known

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0378-4347/01/$ – see front matter
PII: S0378-4347(01)00060-3

quantification problems of the ESI ion source [6] led, despite the use of an internal standard, to relatively high C.V.s of ~5–14% over the calibration range. Chromatographic assays for the determination of clindamycin in bone tissues have not been published so far, to our knowledge. The studies concerning bone tissue concentration of clindamycin have utilized bio-assays for the concentration measurements [1,7,8]. These assays suffer from some methodological and practical problems [9] and are prone to incorrect results due to contamination of the samples by other antibiotics or active metabolites.

In this paper, we describe the determination of clindamycin from human serum and bone tissue with minimal sample preparation using HPLC–atmospheric pressure chemical ionization (APCI)–mass spectrometry. The method is very sensitive and selective with very good precision data for both serum and bone tissue. In the case of bone tissue, only very small samples of ~50 mg are needed.

2. Experimental

2.1. Instrumentation

The HPLC part of the analytical system consists of a Hewlett-Packard HP1100 system (Waldbronn, Germany) comprising a degasser, a binary pump, an autosampler and a thermostated column compartment, controlled by an HP1100 control module. The chromatographic separation of the analytes took place in a Merck Supersphere RP-18 endcapped (particle size 5 μm) 125×4-mm column, equipped with a LiChroCART 4-4 RP-18 guard column (Merck Darmstadt, Germany). The analytes were detected by a ThermoQuest/Finnigan LCQ ion trap mass spectrometer (ThermoQuest, San Jose, USA) equipped with an APCI source. Data were collected and analyzed by the ThermoQuest/Finnigan Xcalibur software package, revision 1.1.

For crushing the bone tissue samples, a special device depicted in Fig. 1 was used. The homogenization of these samples was carried out with an Ultra Turrax T8 homogenizer (IKA Labortechnik, Staufen, Germany).

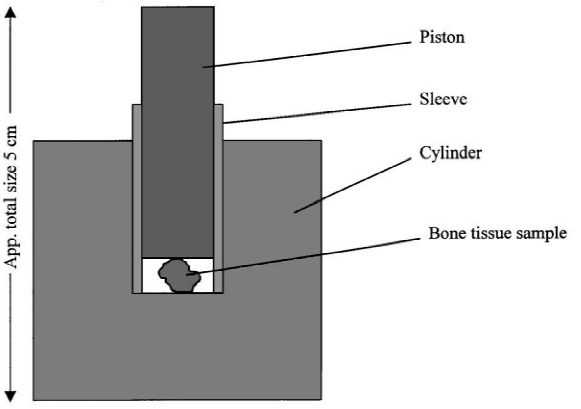

Fig. 1. Bone crushing device.

2.2. Chemicals

Clindamycin-hydrochloride was purchased as pure substance form Pharmacia & Upjohn (Erlangen, Germany). The internal standard lincomycin was used as its hydrochloride soluted in water (one ampoule Albiotic® containing 3.402 g lincomycin-hydrochloride-1 H_2O, i.e. 3.0 g lincomycin free base; Pharmacia & Upjohn, Erlangen, Germany). Trifluoroacetic acid was obtained from Riedel-de Haën (Seelze, Germany), ultra-gradient grade acetonitrile from Baker (Gross-Gerau, Germany). Ultra pure water was produced in our laboratory by a Barnstead EASYpure UV system (Werner, Leverkusen, Germany).

2.3. Sample collection

Bone tissue samples of ~50 mg were obtained in the course of dental operations and were immediately frozen at −20°C until analysis. Blood samples of ~5 ml were drawn form the cubital vein, and serum was separated from blood cells by centrifugation at 2400 *g* for 10 min and frozen at −20°C until analysis.

2.4. Sample preparation for bone tissue

To crush the bone samples to powder a special device depicted in Fig. 1 is applied. The whole device and the bone samples (~50 mg) were cooled in liquid nitrogen to −196°C and hitting the piston

with a hammer produced the bone powder. This powder was weighed and filled into a 1.5-ml micro test tube (Eppendorf, Hamburg, Germany). To the sample, 250 μl of acetonitrile and 300 μl of 0.02% v/v trifluoroacetic acid in water and 50 μl of the I.S. solution (5 μg/ml lincomycin in water) were added (in the case of calibration samples (Section 2.6), only 250 μl of the 0.02% v/v trifluoroacetic acid solution were added to compensate for the volume of the spike solution). This mixture was homogenized with the Ultra Turrax for 1 min at maximum power. After allowing the mixture to equilibrate for 15 min, bone cell fragments were centrifuged at 10 000 *g* for 5 min. Then ~200 μl of the clear extract were transferred into vials for the HPLC autosampler.

2.5. Sample preparation for serum

To 1 ml patient's serum 50 μl of the I.S. solution (100 μg/ml lincomycin in water) were added, 500 μl of this mixture was transferred into a 1.5-ml micro test tube and 500 μl acetonitrile were added. The sample was mixed throughly and allowed to stand for 20 min to complete the protein precipitation. After centrifugation at 10 000 *g* for 5 min, ~200 μl of the clear supernatant were transferred into vials for the HPLC autosampler.

2.6. Bone tissue calibration samples

For calibration purposes, drug free pig bone tissue samples were used. To six samples each containing 50 mg of the powdered bone tissue (Section 2.4), 10, 20 and 50 μl of a 0.5-μg/ml clindamycin in water solution and 10, 20 and 40 μl of a 5-μg/ml clindamycin in water solution were added. Up to 40 μl water was added to yield a total water volume of 50 μl per sample. These calibration samples cover a concentration range from 0.1 to 4 μg/g.

2.7. Serum calibration

The six calibration samples were prepared as follows. Drug free human serum was added up to a final volume of 1 ml to 10, 20 and 50 μl from a solution of 10 μg/ml clindamycin in water and to 10, 20 and 40 μl from a solution of 100 μg/ml clindamycin in water. These calibration samples cover a concentration range from 0.1 to 4 μg/ml.

2.8. Chromatographic conditions and MS-detector settings

For both serum and bone tissue extracts virtually the same chromatographic conditions were applied. Briefly, 10 μl in the case of serum or 20 μl in the case of bone tissue were injected into the chromatographic system and the chromatograms were produced by isocratic elution with 60% trifluoroacetic acid 0.02% v/v in water and 40% v/v acetonitrile at a flow-rate of 0.75 ml/min. The column temperature was maintained at 35°C.

The settings for the APCI ion source were as follows. The vaporizer temperature was set to 450°C, the capillary temperature to 200°C, the capillary voltage was set to 9 V and a discharge current of 5 μA was applied. The sheath gas and the auxiliary gas flow-rates were set to 40 and 10 U (~0.6 and 3 l/min), respectively. The APCI source was working in the ''positive mode'', producing positive charged ions, normally in the form of $[H^+]$ adduct ions. From the ions generated in this way, the mass-charge ratios of 405 and 425 m/z were monitored in the single ion monitoring mode of the ion trap detector, referring to lincomycin and clindamycin, respectively.

3. Results and discussion

3.1. Sample preparation

In the case of serum, the sample preparation is very easy and includes only the addition of the I.S. and the precipitation of the proteins with acetonitrile. Since there is no extraction, an extraction yield is not defined. The only reason for using the quite large amount of 1 ml serum per sample is easy handling in the laboratory. It can be scaled down to ~20 μl, but then the precise addition of the I.S. is difficult.

The crucial point in the preparation of bone tissue samples is the homogenization. The tissue fragments must be as small as possible for a rapid and complete extraction of the analytes into the liquid phase [10]. This is achieved by homogenizing the tissues with

the Ultra Turrax in the extraction liquid. Since the Ultra Turrax is not capable of handling solid bone pieces larger than ~1 mm, the samples have to be crushed prior to the homogenization with the described crushing device. The obtained extraction yield is 84.8% for the I.S. lincomycin and 87.9% for clindamycin.

3.2. Chromatography and mass spectrometry

Some typical chromatograms of serum extracts are shown in Fig. 2 and of bone tissue extracts in Fig. 3. The I.S. lincomycin elutes at 2.2 min and clindamycin at 3.7 min in sharp and symmetrical peaks. Because of the higher injection volume and the different sample composition the retention times in the case of bone tissue are 2.1 and 3.5 min, respectively. These retention time shifts are not optimal but unavoidable due to the unbuffered mobile phase, which in turn has to be used for optimal ionization results in the APCI source of the mass spectrometer.

The monitored mass-charge ratios of 407 and 425 m/z refer to the $[H^+]$ adducts of unfragmented lincomycin and clindamycin. No sodium adducts, dimers or fragments of the analytes are formed in the ion source. Despite the non-sophisticated sample preparation, no peaks from endogenous substances can be observed in the blank sample extracts, either

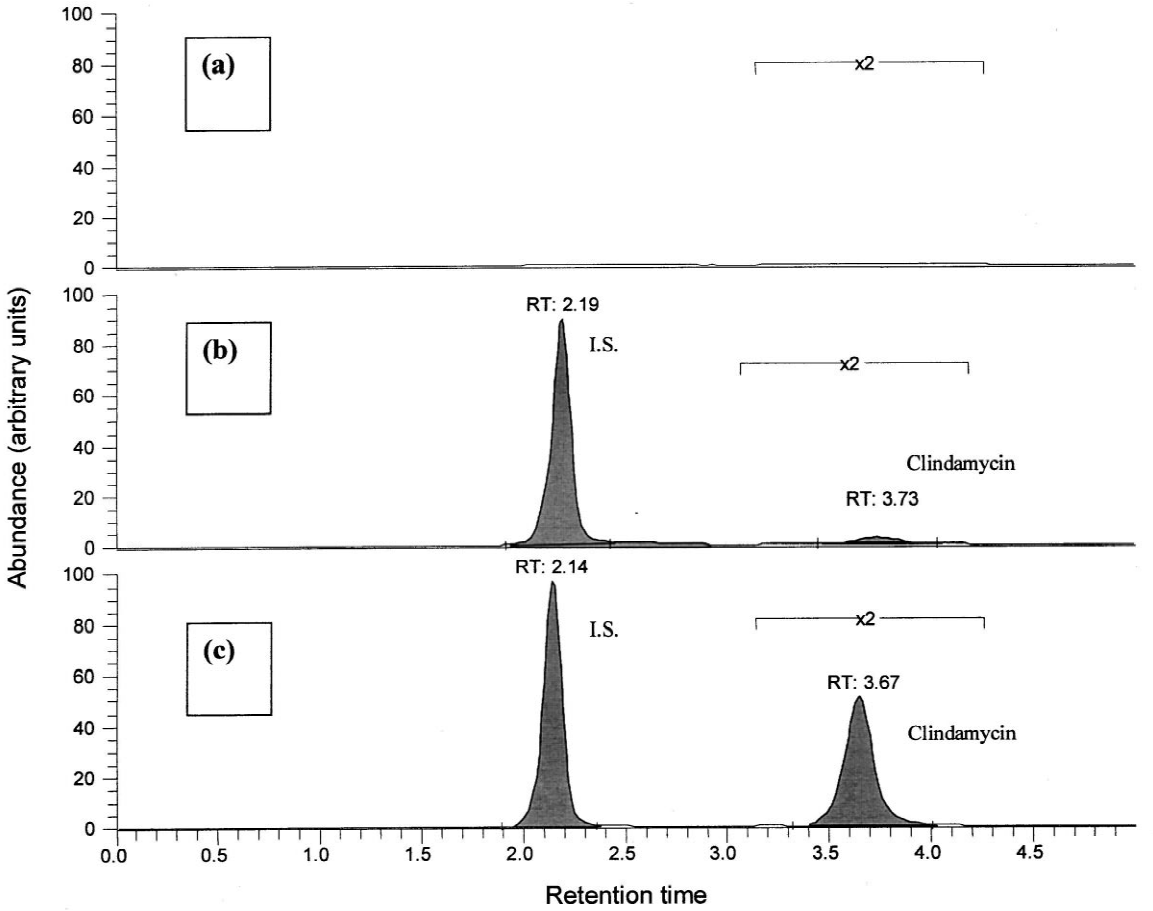

Fig. 2. Typical chromatograms from human serum extracts: (a) drug free human serum, (b) human serum spiked with lincomycin (I.S.) and 0.1 μg/ml clindamycin, (c) patient sample plus I.S. containing 1.51 μg/ml clindamycin.

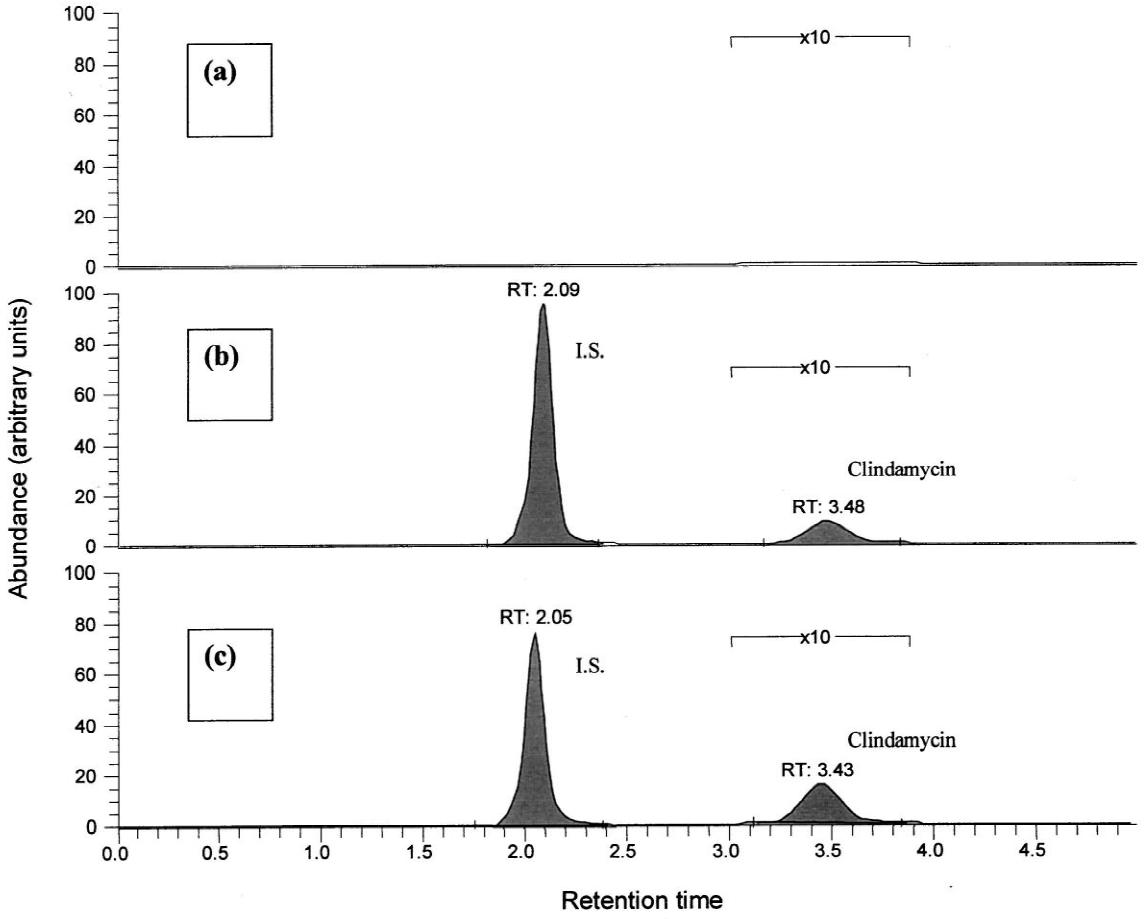

Fig. 3. Typical chromatograms from bone tissue extracts: (a) drug free pig bone tissue, (b) pig bone tissue spiked with lincomycin (I.S.) and 0.1 μg/g clindamycin, (c) patient bone tissue sample (60.8 mg) plus I.S. containing 0.20 μg/g clindamycin.

from serum or from bone tissue. This is a nice illustration of the selectivity of an LC–MS-detector. A further enhancement of the selectivity by applying collision induced fragmentation of the primary ions with single reaction monitoring of the fragment ions [5] does not seems to be required. Also, it is not necessary to improve the signal-to-noise ratio by suppressing chemical background by this technique, because the sensitivity is high enough to cover the relevant concentration ranges. On the other hand, collision induced fragmentation in an ion trap takes time, leading to fewer measurement points during the elution time of a chromatographic peak with implications for the quantification results.

3.3. Calibration and limits of detection and quantification

The calibration ranges of 0.1–4 μg/ml for serum and 0.1–4 μg/g for bone tissue cover the concentrations which can be expected in the course of a normal treatment. The calibration functions are linear in both cases with the parameters ($n=6$) slope = $0.258 \pm 4.10 \times 10^{-3}$, intercept = $6.93 \times 10^{-3} \pm 7.72 \times 10^{-3}$ and $r=0.999$ for serum extracts and ($n=6$) slope = $0.158 \pm 2.59 \times 10^{-3}$, intercept = $4.24 \times 10^{-3} \pm 4.87 \times 10^{-3}$ and $r=0.999$ for the bone tissue extracts.

The limit of detection for clindamycin from serum

Table 1
Intra-day precision and accuracy

Matrix	Concentration level	Measured concentration (average)	Unit	RSD (%)	Accuracy (%)	*n*
Serum	0.10	0.11	μg/ml	4.48	+7.04	10
Serum	1.00	1.00	μg/ml	2.23	−4.93	6
Serum	4.00	4.20	μg/ml	1.87	+4.95	10
Bone tissue	0.10	0.09	μg/g	8.41	−13.3	10
Bone tissue	1.00	1.00	μg/g	3.01	−0.22	6
Bone tissue	4.00	3.53	μg/g	5.87	−11.8	10

samples is 20 ng/ml and from bone tissue samples (50 mg) 30 ng/g at an S/N ratio larger than 3. The limits of quantification are set for serum and bone tissue samples to the lowest calibration levels of 0.1 μg/ml (RSD 4.5%) and 0.1 μg/g (RSD 8.4%), respectively. Regarding the quite low RSDs at the given limits, lower limits of quantification may be possible, but they are situated outside the calibration ranges, which in turn cover the relevant concentrations of clindamycin in serum and bone tissue in pharmacokinetic investigations.

3.4. Precision and accuracy

Data regarding the intra- and inter-day precisions and accuracies of the assays are summarized in Tables 1 and 2, respectively. The precision of the serum- and bone-tissue assays are satisfying and illustrate the stability of the ion-forming process in an APCI source. Only the inter-day RSDs at the lowest concentration levels for both assays and the inter- and intra-day accuracy deviations for the bone tissue assay at the lowest concentration level are quite high. Nevertheless, the precision and accuracy data for both assays are within acceptable limits.

4. Conclusion

The described procedure for the determination of clindamycin in serum samples is fast and easy. Due to the sensitive and selective MS-detector only very little sample preparation is needed. The very good precision of the assay reflects the stable ion-forming process in an APCI ion-source, which, in cases where great sensitivity is not needed, is superior to an ESI ion-source. The procedure for the determination of clindamycin in bone tissue samples is the first assay described in the literature which applies a chromatographic system. It is fast and easy as well, and it is sensitive enough to be employed on sample sizes as low as ~50 mg. Therefore, it is applicable to human bone tissue samples yielded in the course of dental operations.

Acknowledgements

The authors would like to thank Professor F. Kees (Department of Pharmacology and Clinical Pharmacology, University of Regensburg, Germany) for valuable advice and the construction plan of the bone

Table 2
Inter-day precision and accuracy

Matrix	Concentration level	Measured concentration (average)	Unit	RSD (%)	Accuracy (%)	*n*
Serum	0.10	0.10	μg/ml	11.93	−1.13	6
Serum	1.00	0.99	μg/ml	3.46	−1.03	6
Serum	4.00	3.82	μg/ml	2.08	−4.57	6
Bone tissue	0.10	0.09	μg/g	10.77	−12.19	6
Bone tissue	1.00	1.01	μg/g	3.20	+0.60	6
Bone tissue	4.00	4.01	μg/g	2.36	+0.36	6

crushing device and G. Bethke, MD (Center of Dentistry, Humboldt-University, Berlin, Germany) for the collecting and preparing the human bone tissue samples.

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