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Journal of Chromatography A, 804 (1998) 211–215

JOURNAL OF CHROMATOGRAPHY A

Short communication

Use of ion chromatography as an alternative method for the analysis of calcium in calcium mupirocin

J.P. Waterworth[a,*], L.R. Skinner[b]

[a]*Pharmaceutical Technologies, SmithKline Beecham Pharmaceuticals, New Frontiers Science Park (South), Third Avenue, Harlow, Essex, CM19 5AW, UK*

[b]*Division of Chemistry, University of Hertfordshire, Hatfield Campus, College Lane, Hatfield, Hertfordshire, AL10 9AB, UK*

Abstract

Ion chromatography was investigated as an alternative technique to atomic absorption for the determination of calcium in calcium mupirocin drug substance (calcium pseudomonate dihydrate). The analytical method was evaluated by generating data on the parameters of specificity, linearity, precision, accuracy, sensitivity, robustness and stability of solution. The validation exercise shows that ion chromatography is a viable alternative to atomic absorption for the analysis of counter-ions in mupirocin. © 1998 Elsevier Science B.V.

Keywords: Calcium; Inorganic cations; Enzyme inhibitors; Calcium mupirocin; Antibiotics

1. Introduction

Calcium mupirocin (calcium pseudomonate dihydrate) is an isoleucyl tRNA synthetase inhibitor. It is the active component in a topical broad-spectrum antibiotic ointment for bacterial skin infections such as impetigo, folliculitis and furunculosis [1]. Purity is determined by analysis of the mupirocin free acid by reverse phase liquid chromatography [2]. Calcium content has been determined in the past by atomic absorption spectroscopy (AAS) using a nitrous oxide–acetylene flame [3]. However, other counter-ions would not be detected using this method.

In recent years ion chromatography (IC) has become a powerful alternative technique for the determination of inorganic ions [4–7]. IC was thought to be a viable alternative technique to AAS. It would also give information on other salts, such as sodium mupirocin, which may be formed during synthesis.

Also, IC would be less labour intensive as an autosampler would be used, allowing a larger number of samples to be analysed in a given time.

This work validates the IC method as an alternative technique for the determination of calcium content in calcium mupirocin.

2. Experimental

2.1. Reagents

All chemicals were of analytical reagent grade and all reagents, eluents and standard solutions were prepared using purified water (Ph. Eur) [8].

$CaCl_2 \cdot 2H_2O$ (Analar, BDH–Merck, Dorset, UK) was used to prepare cation stock standard solutions.

Methanesulphonic acid (puriss grade, Fluka,

*Corresponding author.

0021-9673/98/$19.00
PII S0021-9673(97)01247-8

Buchs, Switzerland) was used to prepare mobile phase for the separation.

2.2. Plasticware

All analyses were carried out using pre-washed polypropylene flasks and vials.

2.3. Instrumentation

A Dionex DX500 ion chromatograph with a Dionex GP40 gradient pump, Dionex ED40 electrochemical detector (in conductivity mode), a Thermo Separation Spectra SYSTEM AS3500 autosampler, a Rheodyne injection valve and a 100-μl sample loop were used. All flow paths in the system were constructed from chemically inert, metal-free polydietherketone (PEEK).

Separation was achieved with Dionex CS12 analytical (4 mm I.D.) and CG12 guard columns. Suppression was given by a Dionex Cation self-regenerating suppressor (CSRS-1, 4 mm).

Area data were collected and analysed using a Perkin–Elmer Access Chrom data handling package.

2.4. Analytical method

2.4.1. Standard preparation

Standard solutions were prepared freshly for each analysis. Approximately 1.46 g of $CaCl_2 \cdot 2H_2O$ was accurately weighed into a 1000-ml volumetric flask. Flasks were made up to volume with purified water. The solution was diluted 10.0 ml to 1000-ml with purified water.

2.4.2. Sample preparation

Approximately 54 mg was accurately weighed into a 100-ml flask. A 10-ml volume of methanol was added and the flask sonicated for 2 min to dissolve the sample. The flask was made to volume with purified water.

2.4.3. Chromatographic conditions

Calcium was eluted using 20 m*M* methanesulphonic acid as mobile phase with a flow-rate 1.5 ml min^{-1}. A 100-μl injection volume was used with a run time of 10 min. The conductivity detector range was 50 μS. See Fig. 1.

2.5. Analyses

Validation based on International Conference on Harmonisation (ICH) guidelines [9] and own laboratory guidelines [10] was carried out on the IC method, with responses for peak area collected and evaluated.

2.5.1. Specificity

A solution containing ammonium (1.6 ppm), lithium (0.2 ppm), magnesium (0.8 ppm), potassium (0.8 ppm), sodium (0.8 ppm), together with calcium (4 ppm) was separated by the cation method to show that the component of interest was resolved.

2.5.2. Linearity

Five calcium standards (in duplicate) were prepared covering a concentration range of 25% to 150% of the expected calcium concentration.

2.5.3. Precision

(1) Repeatability: A sample of the drug was prepared at the expected calcium concentration and analysed six times on the same day, using the same equipment, in the same laboratory, by the same analyst.

(2) Intermediate precision: A sample of the drug was prepared at the expected calcium concentration and analysed in duplicate on six different days by different analysts using different chromatography columns. As only one IC for each analysis was available it was not possible to perform the analyses on different instruments and in different locations.

2.5.4. Accuracy

Three solutions were prepared accurately at approximately 90%, 100% and 110% of expected sample calcium concentrations. A recovery exercise was then performed. This was performed in duplicate.

2.5.5. Limits of detection and quantification

Detection limit (DL) and quantification limit (QL) were determined by performing a linearity exercise covering a range of concentrations close to the detection limit. All solutions (including purified water as a blank solution) were injected six times and a mean response was taken. A computer package

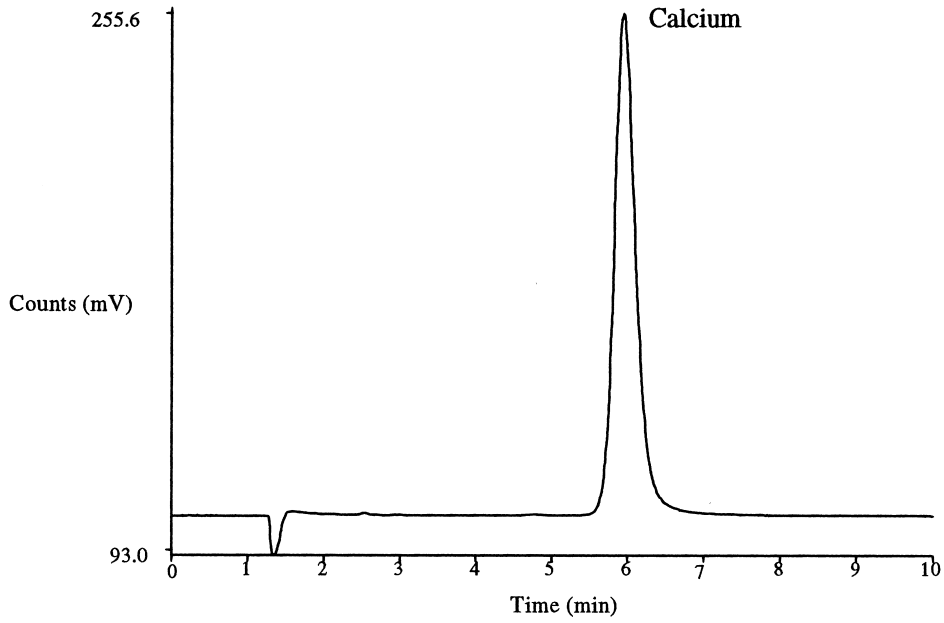

Fig. 1. Sample chromatogram.

(Statistica) was utilised to plot concentration versus detector response and calculate the DL and QL using the following formulae:

$$\text{Limit of detection} = \frac{3Sx/y}{b}$$

$$\text{Limit of quantification} = \frac{10Sx/y}{b}$$

where b = slope of best fit regression line; Sx/y = estimate of residual standard deviation. Further details of this calculation are available on request from the author.

2.5.6. Robustness

Flow-rate and concentration of the eluent were varied by ±10% of the values quoted in the method. Old versus new separation columns were evaluated. The effects on retention times were noted. The ion chromatograph was located in a temperature controlled laboratory. Separation columns, suppressors and detector cell were housed in an insulated Dionex LC20 chromatography enclosure therefore effects of changes in room temperature were deemed to be insignificant.

2.5.7. Stability in container

The stability of a sample solution (stored in polypropylene flasks at ambient temperature) were evaluated by determining whether there was a change in assay over a period of days. Results from the method reproducibility exercise were used.

3. Results and discussion

In order to determine whether a method is suitable for a particular use, the validation data generated

Table 1
Comparison of data obtained by three analytical techniques

Batch	Atomic absorption spectroscopy (AAS)	Ion chromatography (IC)	Inductively-coupled plasma emission spectroscopy (ICP)
1	2.6%	3.67%	3.6%
2	2.7%	3.66%	3.6%
3	2.8%	3.56%	3.5%
4	2.8%	3.57%	3.4%
Mean	2.7%	3.62%	3.5%
R.S.D.	3.5%	1.6%	2.7%

The theoretical quantity of calcium in calcium mupirocin is 3.72% by mass.

Table 2
Summary of cation validation results by area response

	Calcium
Detection limit	0.05 ppm
Quantification limit	0.15 ppm
Linearity (5 data points)	$y = 7.389 \cdot 10^5\, x - 7.952 \cdot 10^4$
Correlation coefficient	0.9972
p-value	0.056
Repeatability	
Mean ($n = 6$)	4.00 ppm
R.S.D.	0.62%
Intermediate precision	
Mean ($n = 6$)	4.05 ppm
R.S.D.	1.4%
Accuracy	
Recovery ($n = 6$)	99.84%
R.S.D.	1.3%
Stability in Container at ambient temperature	>7 days

using that method must meet pre-determined acceptance criteria. In this case, ICH guidelines for the evaluation of medicinal products were used. The acceptance criteria for a change from existing AAS to new IC methodology were that the IC method should be accurate, equal to or more sensitive and precise than the associated AAS method. The data generated by IC were compared to the data generated on the same samples by both the atomic absorption method and by the more sensitive inductively coupled plasma (ICP) technique. See Table 1.

The theoretical quantity of calcium in calcium mupirocin is 3.72% by mass. The IC data and the ICP data were comparable and close to the theoretical calcium content. The AAS results were approximately 25% below the nominal value. This could possibly be due to the calcium being ionised in the nitrous oxide–acetylene flame.

3.1. Method validation

A summary of results from the validation exercises is given in Table 2.

The methodology was shown to be specific for lithium, sodium, ammonium, potassium, magnesium and calcium ions. No interference was noted between

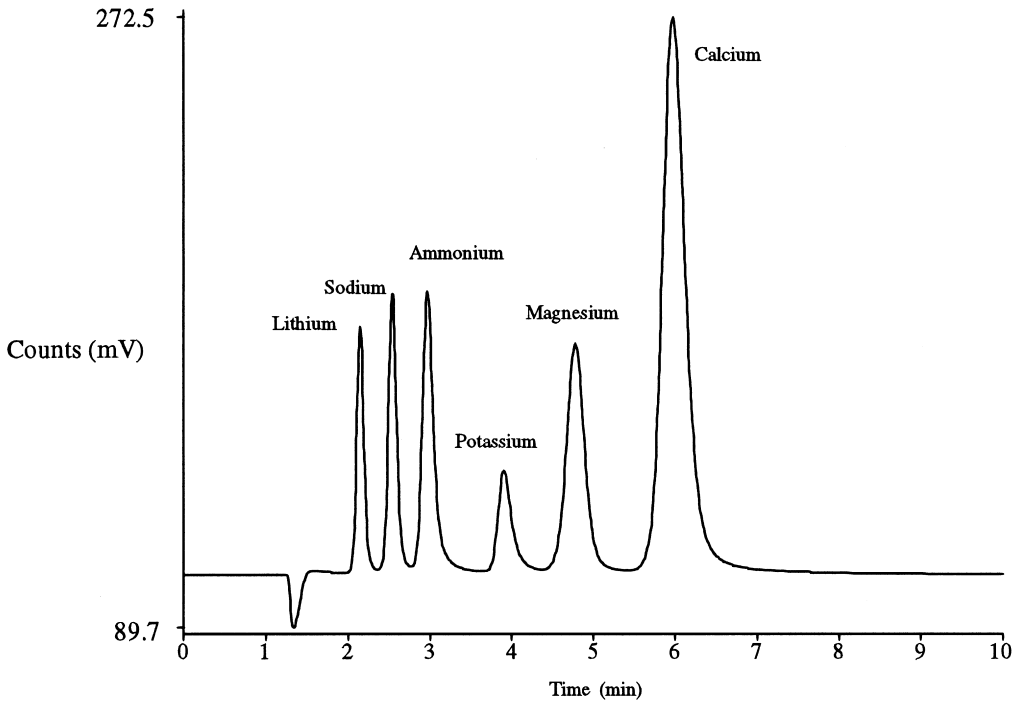

Fig. 2. Specificity.

the calcium ion and the other ions investigated. See Fig. 2.

The response is linear up to at least 150% of expected concentration with the plot passing through the origin with 95% confidence. The method is robust with regard to mobile phase composition, flow-rate and separation columns. Modification of the methanesulphonic acid concentration by ±10% varied the retention time of calcium between 5.2 to 7.1 min. Altering the flow-rate by ±10% varied the retention time between 5.5 to 6.6 min. Evaluation of old vs. new separation columns showed no change in the retention time of the calcium peak. In all three cases the elution order of lithium, sodium, ammonium, potassium, magnesium and calcium remained unchanged with resolution between all the peaks.

4. Conclusions

IC is shown to be superior to atomic absorption spectroscopy, particularly with respect to accuracy, in the determination of the calcium content of calcium mupirocin. Other ions, including sodium, may also be monitored at the same time.

The validation data shows that the method is suitable for use for quantitative analysis of calcium in calcium mupirocin by area response.

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