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Short Communication

A reversed-phase high-performance liquid chromatographic method for the determination and identification of clarithromycin as the drug substance and in various dosage forms

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Introduction

Clarithromycin (Fig. 1) is a semi-synthetic antibiotic which was discovered by Taisho Pharmaceuticals and is being developed by Taisho and Abbott Laboratories. After synthesizing clarithromycin from erythromycin A, the drug substance is to be formulated into various products, including tablets, injectables and oral paediatric suspensions.

A microbiological assay for clarithromycin has been reported for biological matrices [1], but it is inappropriate for drug products because of its poor precision and lack of selectivity [2, 3]. High-performance liquid chromatography (HPLC) is routinely used for the selective and accurate determination of the active constituents in pharmaceutical matrices [4]. HPLC is also routinely used in pharmacokinetics studies and several procedures for the determination of clarithromycin in biological samples have been reported [5–7]. However, a precise and accurate potency procedure for clarithromycin in any pharmaceutical matrix has not been reported. In addition to precision and accuracy, the ideal analytical method would also be stability indicating and would be able to identify and quantitate all possible related substances. To accomplish all these requirements in a single chromatographic assay, the analysis times would be impractically long. Thus to simplify testing, a combination of two assays has been used to control completely the final drug product. A simple, accurate and precise stability indicating assay has been developed and is presented here. An additional related substance assay has been used to identify and quantitate individual impurities in clarithromycin [8].

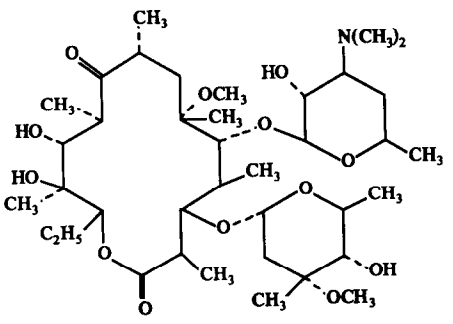

Figure 1
Structure of clarithromycin.

Experimental

Equipment

The basic HPLC system consisted of a Spectra-Physics Model SP-8700 (Spectra-Physics, San Jose, CA, USA) high-pressure pump and an IBM Model LC/9505 autosampler (IBM Instruments Inc., Danbury, CT,

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USA). The detection was achieved with a Kratos Spectroflow 783 (Kratos Analytical Instruments, Ramsey, NJ, USA). The separation was performed on a column (150 × 4.6 mm, i.d.) containing Nucleosil C_{18} (5 μm, Alltech Associates Inc., Deerfield, IL, USA). Column temperature was controlled by using a Shimadzu Model CTO-6A oven (Shimadzu Corp., Tokyo, Japan). The chromatographic peaks were digitized, integrated and recorded using a Spectra-Physics Model 4270 computing integrator equipped with a Spectra-Physics WINner data processing system.

Chromatographic conditions

The analysis was performed using a 1 ml min^{-1} flow of methanol–KH_2PO_4 (0.067 M) (60:40) at an apparent pH* of 3.5. The pH* adjustment was made using phosphoric acid. The separation of clarithromycin from degradation products, related substances, formulation excipients and the internal standard was obtained using a Nucleosil C_{18} column maintained at 50°C. The analytes were detected at 210 nm with a typical range of 0.04 AU/10 mV with a rise time of 2.0 s. An injection volume of 50 μl was used for all formulations. The working standard solution was mobile phase containing clarithromycin at about 410 μg ml^{-1} and the internal standard, biphenyl, at approximately 2.6 μg ml^{-1}. This working standard solution was filtered through the same type of membrane as the sample prior to injection.

The above conditions were used for all samples except for the hydroxypropylmethylcellulose (HPMC) suspensions. For the HPMC suspensions, the following changes were made: (1) the eluent was acetonitrile–KH_2PO_4 (0.067 M) (65:35) with an apparent pH* of 3.5 at 1.5 ml min^{-1} and (2) the internal standard was butylparaben, Baker NF grade, at a concentration of approximately 7.2 μg ml^{-1}.

Sample preparation

Bulk drug. An accurately weighed amount of clarithromycin was dissolved in methanol to obtain a 2 mg ml^{-1} solution. Then, 10 ml of this solution was added to a constant amount of internal standard and diluted with mobile phase to 50.0 ml. The resulting solution was filtered through a 0.4-μm polycarbonate membrane prior to injection.

Capsules. The contents of 10 capsules were blended and the mean fill weight determined. Based on the mean fill weight and the label claim, an amount equivalent to 200 mg of theoretical clarithromycin activity was added to a 100-ml volumetric flask containing 70 ml of methanol. After a 30-min shake extraction, the solution was diluted with methanol to 100 ml. This solution was then mixed with an appropriate amount of internal standard and diluted with mobile phase to a concentration of approximately 400 μg ml^{-1}. Prior to injection, the sample solution was filtered through a 0.4-μm polycarbonate membrane.

250 mg Tablets. Five intact tablets were added to 50 ml of water in a 500-ml volumetric flask and shaken until the tablets completely disintegrated. The clarithromycin was then extracted by shaking for 30 min in approximately 300 ml of methanol and diluted to 500 ml with methanol. After the excipients settled, 10 ml of the supernatant was mixed with internal standard and diluted with mobile phase to 50 ml. This solution was filtered through a 0.4-μm polycarbonate membrane prior to injection.

500 mg Tablets. In a glass mortar and pestle, four tablets were ground to a fine powder and quantitatively transferred with methanol into a 500-ml volumetric flask. The clarithromycin was extracted with approximately 300 ml of methanol by shaking for 30 min and then diluted with methanol. Upon settling of the excipients, 5.0 ml of the supernatant was mixed with internal standard and diluted with mobile phase to 50.0 ml. This solution was filtered through a 0.4-μm polycarbonate membrane prior to injection.

Injectable clarithromycin (500 mg vials). Each vial was reconstituted with sterile water as described on the label. A disposable syringe and needle was used to transfer the withdrawable contents into a 250-ml volumetric flask and then diluted with water. A 10-ml aliquot of this solution was mixed with internal standard and diluted with mobile phase to 50 ml. After filtration through a 0.4-μm polycarbonate membrane, chromatographic analysis was performed. For other experimental dose units, appropriate dilutions were made in order to obtain the same theoretical injection concentration.

Clarithromycin oral suspension. For 125 mg doses, 2 dose units were measured with a disposable syringe and transferred to a 500-ml volumetric flask. The solution was shaken mechanically for 30 min in 200 ml of K_2HPO_4 buffer (0.067 M). After dispersing, about 200 ml of methanol was added and shaken for an additional 30 min. The solution was diluted with methanol to 500 ml and filtered through a 0.4-μm nylon membrane prior to injection. For other experimental formulations, modifications were made to obtain the same theoretical concentrations.

Hydroxypropyl methylcellulose suspensions. The suspensions were thoroughly mixed by either stirring or sonicating. An aliquot of the suspension was then mixed with the internal standard, butylparaben, and diluted until the final concentration was between 300–500 μg ml^{-1}. This solution was then filtered through a polycarbonate membrane prior to injection.

Results and Discussion

Analysis of formulations

In these laboratories, it is preferred to use an internal standard for potency assays because of the increased precision it provides for the method. Care must be taken in choosing an internal standard so that any related substances or possible manufacturing impurities do not interfere. Biphenyl was chosen as the internal standard because it met this requirement. Figure 2 illustrates a typical chromatogram of the standard and blank. The same chromatographic conditions were maintained for all formulations except for the oral suspensions. No internal standard was used with the paediatric formulations because the sample matrix interfered with the internal standard. Otherwise, the chromatography for the paediatric formulations was unchanged. To prove that the method was stability indicating, clarithromycin was degraded with aqueous HCl (0.1 M) methanolic sodium hydroxide (0.1 M), high intensity UV radiation and hydrogen peroxide. Chromatograms of degraded clarithromycin are shown in Figs 3–6. None of the degradation conditions produced any products which interfered with biphenyl or clarithromycin. Therefore, the assay could be used to monitor clarithromycin stability.

Analysis of the hydroxypropyl methylcellulose suspensions

Concurrently, with the development of the methanol eluent system for the bulk drug and the first experimental formulations, a second chromatographic system using an acetonitrile-based eluent was being evaluated for use in HPMC suspensions. A typical chromatogram of the standard preparation is shown in Fig. 7. A comparison of Figs 2 and 7 shows that the acetonitrile eluent system has a much shorter

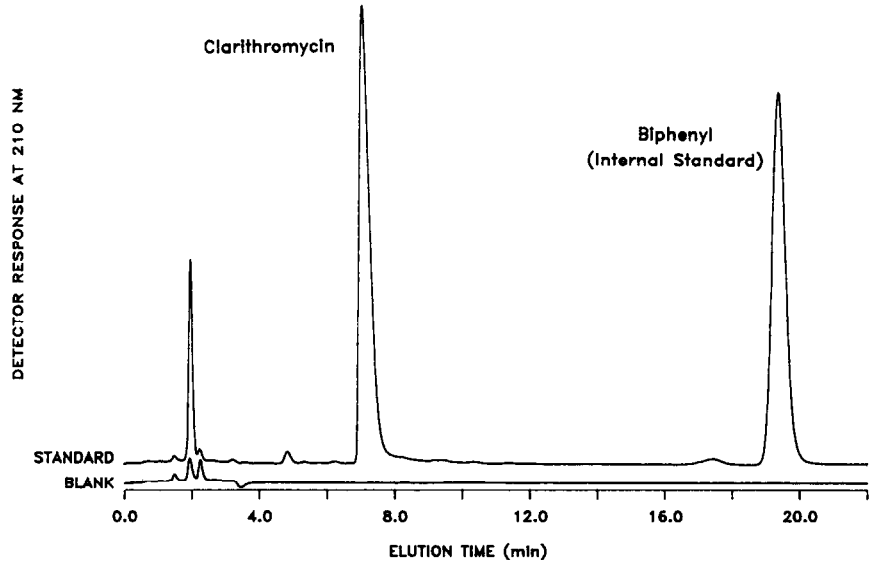

Figure 2
Typical chromatograms of a blank and a working standard solution. A methanol–0.067 M KH_2PO_4 (60:40) eluent at 1 ml min^{-1}, 50°C with a Nucleosil C_{18} column results in good separation of clarithromycin (7.5 min) and the biphenyl internal standard (19.5 min).

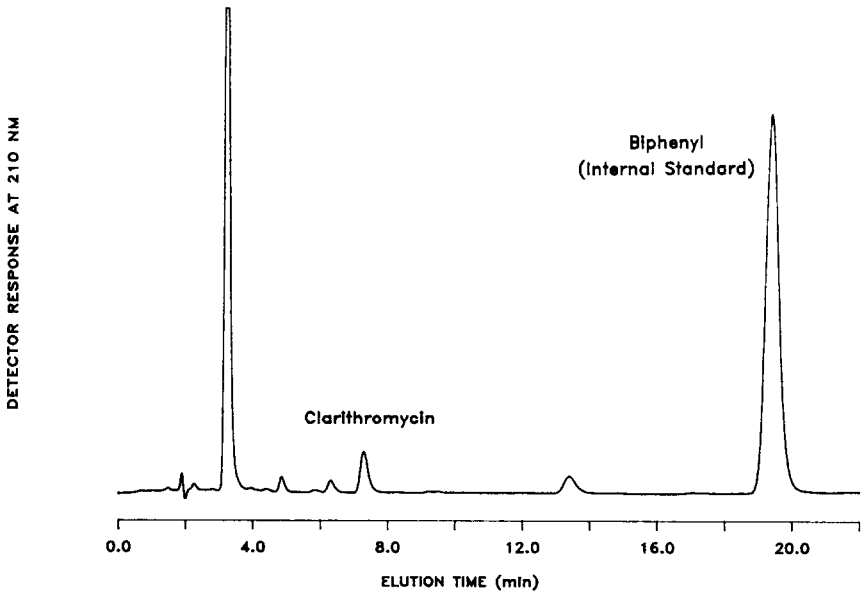

Figure 3
Typical chromatogram of acid degraded clarithromycin. Chromatographic conditions were the same as Fig. 2. Hydrochloric acid at 0.1 M for 30 min was used for degradation.

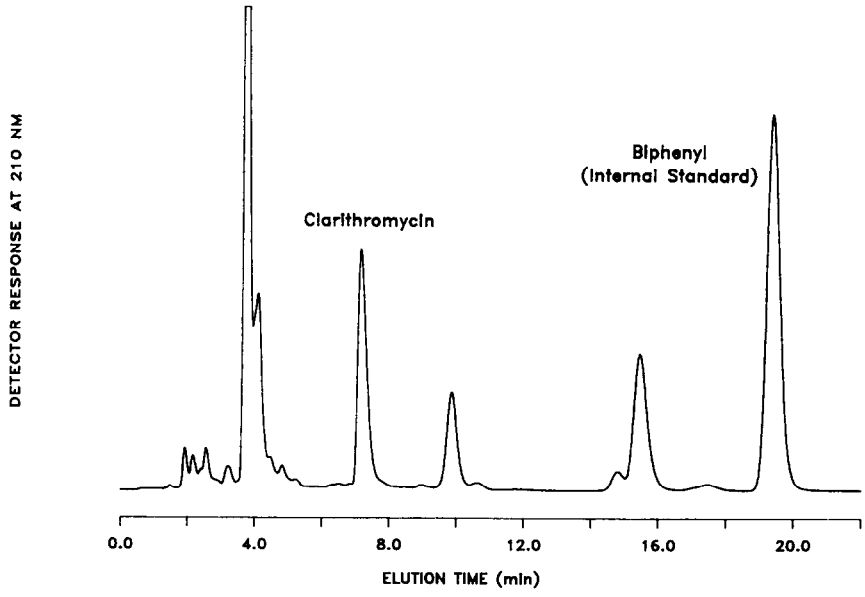

Figure 4
Typical chromatogram of base degraded clarithromycin. Chromatographic conditions were the same as Fig. 2. Methanolic sodium hydroxide at 0.1 M refluxing for 3 h was used for degradation.

run time. Potential degradation products and some of the manufacturing impurities co-eluted with the internal standard in the acetonitrile system. Therefore, this assay would not be totally stability indicating and the methanolic system was selected for validation.

Sample preparation

The initial step of the method validation was to determine the optimum procedure needed to completely remove clarithromycin from the formulation matrix. Since both the bulk drug substance and the injectable formulation were soluble, only a simple dilution was needed. However, the other formulations required selection of a sample dispersion method, an extraction solvent and extraction time. For both capsules and 250-mg tablets, a quanti-

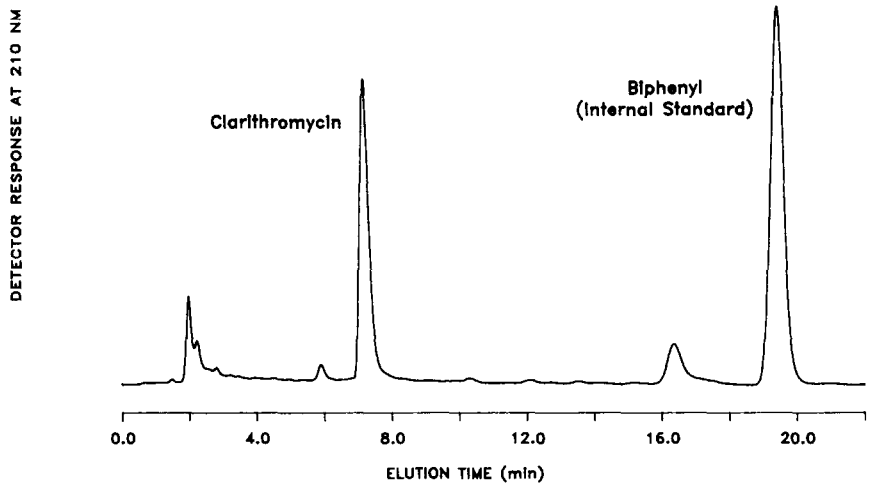

Figure 5
Typical chromatogram of high intensity UV degraded clarithromycin. Chromatographic conditions were the same as Fig. 2. Degradation was produced by exposure of solid clarithromycin to high intensity UV radiation for 3 h.

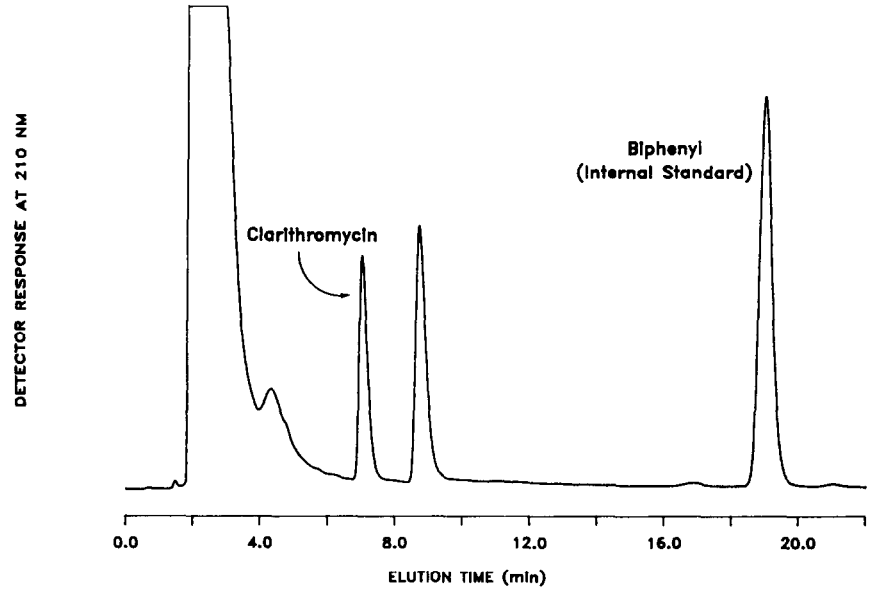

Figure 6
Typical chromatogram of hydrogen peroxide degraded clarithromycin. Chromatographic conditions were the same as Fig. 2. Degradation was produced by mixing a 2 mg ml^{-1} clarithromycin methanolic solution with 9% hydrogen peroxide for 45 min.

tative amount of clarithromycin was extracted by shaking in methanol for 30 min. Because the 500-mg tablet formulation was very similar in composition to the 250-mg formulation, the only change needed was grinding the tablets prior to extraction. However, due to the significant differences in the excipients, the oral suspension required an initial dispersion in aqueous phosphate buffer and was then extracted with a methanol–buffer mixture. Also, due to the limited solubility in aqueous media, the clarithromycin required 60 min for complete extraction.

Linearity

Under the reported conditions, the linear range for the internal standard method extended from 98.6 to 986 $\mu g\ ml^{-1}$ of clarithromycin with a correlation coefficient of 0.9997 and an equation for the least-squares linear regression line of $y = 0.0024x - 0.0077$, where y is peak area ratio and x is concentration. The

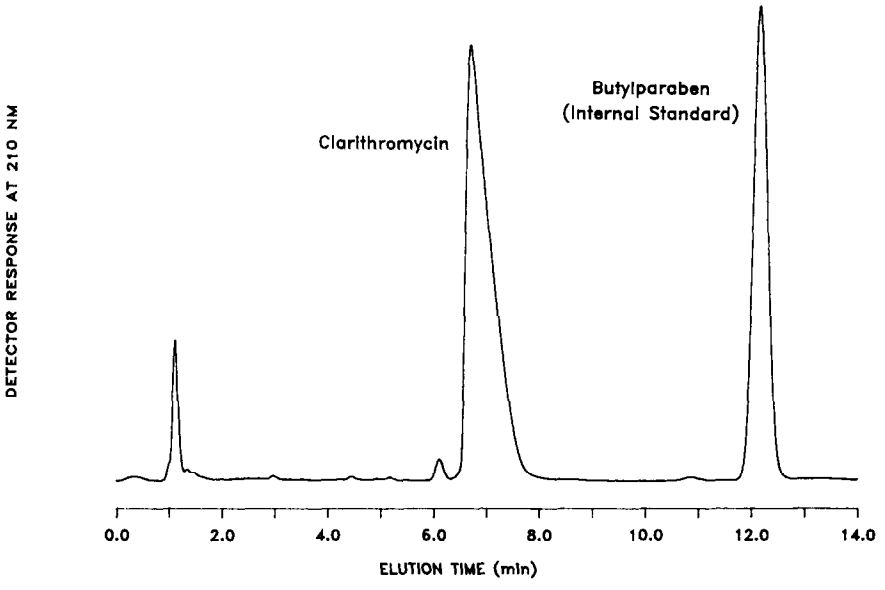

Figure 7
Typical chromatograms of a clarithromycin working standard solution for the acetonitrile eluent system. An acetonitrile–0.067 M KH_2PO_4 (35:65) eluent at 1.5 ml min^{-1}, 50°C with a Nucleosil C_{18} column results in good separation of clarithromycin (7.0 min) and the butylparaben internal standard (12.0 min).

linear range for the external standard method extended from 102 to 817 μg ml^{-1} of clarithromycin with a correlation coefficient of 1.0000 and an equation for the least-squares linear regression line of $y = 112.35x - 0.1750$. For the HPMC suspensions the linear range was from 95.5 to 955 μg ml^{-1} and the equation for the least-squares line was $y = 506.26x - 0.0088$ with a correlation coefficient of 1.0000.

System suitability

The column parameters which were monitored daily to test the system suitability were plate count, capacity factor, tailing factor, resolution and reproducibility. These parameters were obtained by a comparison with previous data from three different departments. The following parameters were established for a suitable system: (1) the plate count was >2100 for clarithromycin, (2) the clarithromycin tailing factor was between 1.0–1.7, (3) the k' value for clarithromycin was >2.4, and (4) the resolution between the clarithromycin and biphenyl peaks must be >16 to ensure adequate resolution of clarithromycin related compounds. To ensure accurate quantitation, the peak response ratio of three consecutive injections had an RSD <3.0%. Every time a chromatographic component was changed, the system suitability was verified.

Accuracy and precision

The accuracy and precision of a method were determined by the technique of standard addition and recovery. This involved adding a known amount of reference clarithromycin to the appropriate placebo. Standard addition and recovery have been performed on all

Table 1
Standard addition and recovery of clarithromycin to capsule placebo formulation

% Age of nominal	Clarithromycin Added (mg)	Found (mg)	Recovery (%)
0	0	0	—
75	150	149	99.3
75	149	145	97.3
100	201	201	100.0
100	198	194	98.0
125	250	251	100.0
125	250	245	98.0

Table 2
Standard addition and recovery of clarithromycin to a 250 mg tablet placebo formulation

% Age of nominal	Clarithromycin Added (mg)	Found (mg)	Recovery (%)
0	0	0	—
50	125	121	96.8
100	250	247	98.8
150	376	370	98.4

Table 3
Standard addition and recovery of clarithromycin to a 500 mg placebo tablet formulation

% Age of nominal	Clarithromycin Added (mg)	Found (mg)	Recovery (%)
0	0	0	—
75	1456	1412	97.0
100	1933	1875	97.0
125	2414	2354	97.5

Table 4
Standard addition and recovery of clarithromycin to an injectable formulation

% Age of nominal	Clarithromycin Added (mg)	Found (mg)	Recovery (%)
100	500	511	102.2
100	500	510	102.0
100	500	516	103.2

Table 5
Standard addition and recovery of clarithromycin to an oral suspension placebo formulation

% Age of nominal	Clarithromycin Added (mg)	Found (mg)	Recovery (%)
80	168	165	98.2
100	212	214	100.9
120	252	254	100.8

formulations (Tables 1–5). The recovery of drug from the various formulation ranged between 96.8–103.0%. This recovery confirmed the accuracy for each matrix and because no drug was found from the placebos; the absence of interference from the matrix was also confirmed.

Ruggedness

After the accuracy of the methods has been proven, the ruggedness of the procedures was tested by having several analysts assay a single lot of a particular formulation using different HPLC equipment and columns. The results of these experiments have been summarized in Table 6. The data indicate for the bulk drug and all of the different formulations, the assay was reproducible with an RSD of about 2.0%. Table 6 also illustrates that under all of the different testing conditions, no bias was detected that could be attributed to an analyst or instrumental conditions.

The method also provides for the identification of clarithromycin throughout the manufacturing process. The retention time of clarithromycin in the sample preparation was required to be within ±2.5% of the average retention time of the standard preparation.

Conclusions

A liquid chromatographic method for the determination of clarithromycin as the bulk substance and the active component in formu-

Table 6
Precision data for clarithromycin activity in bulk drug and various formulations

Bulk		Capsules		250 mg Tablets		500 mg Tablets		Injectables		Oral suspension	
Analysts	Activity ($\mu g\ mg^{-1}$)	Analysts	Activity (mg/cap)	Analysts	Activity (mg/tab)	Analysts	Activity (mg/tab)	Analysts	Activity (mg vial)	Analysts	Activity (mg/dose)
1	966	1	199	1	246	1	484	1	513	1	257
1	972	1	194	1	246	2	496	1	511	1	257
1	967	1	197	2	239	2	496	1	510	2	259
1	989	1	197	2	233	3	504	2	527	2	265
2	943	1	196	1	246	3	499	2	534	2	259
2	930	1	202	1	247			3	538	2	263
3	974	2	204								
3	983	2	191								
		2	200								
Mean	966		198		243		496		522		260
SD	19.8		4.0		5.6		7.0		12.4		3.3
RSD (%)	2.0		2.0		2.3		1.5		2.4		1.3

lations is presented. The method is accurate, precise and rugged enough to be used in several different types of formulations. In conjunction with a related substances analysis, total chromatographic control of the bulk substance and formulations can be maintained.

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