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Determination of content uniformity and distribution characteristics of an investigational drug in its tablets dosage form and granule by ICP-AES

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Abstract

An investigational drug (**A**) in its calcium salt form has been developed as the tablet dosage form. Monitoring drug distribution and uniformity in granules and tablets during early stage formulation/process development is critical for drug product quality control and process robustness. In this report, an efficient and reliable analytical method for monitoring drug compound **A** uniformity and distribution has been developed by analyzing calcium, the counter ion of the drug substance, by Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES). In this method, calcium in compound **A** granule and tablet samples was digested with 1 M hydrochloric acid by heating at 90 °C for 2 h. The resulting suspension was centrifuged, and the supernatant was directly aspirated into an ICP-AES. This method has been validated to demonstrate satisfactory precision, accuracy, specificity and sensitivity. Finally, this method has been used to analyze sieve fraction granules and tablets of drug compound **A**. The data generated were highly comparable to those by validated HPLC methods (UV method can not be applicable due to significant bias). In comparison with HPLC methods, this method demonstrates a significantly improved efficiency with very short analysis time (1 min per sample), and can be used as an excellent alternative for UV and HPLC methods to support formulation screening.

Keywords: ICP-AES; Calcium; Content uniformity; Formulation screening

1. Introduction

Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) has been widely used as a quantitative analysis, characterization, and quality control tool in biomedical [1–4], pharmaceutical [5–7], food industry [8–12] and environment [12–15] for multi-elemental analyses. This technique offers several advantages over the conventional absorbance methods such as wide dynamic range, excellent sensitivity and specificity. With the sampling devices incorporated, the accuracy, precision and efficiency of ICP-AES methods have been significantly improved.

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Investigational drug compound **A** in its calcium salt form is a drug candidate being developed as a tablet dosage form. At the initial formulation screening stage, many formulations and dosage forms were developed and evaluated in terms of processibility and stability within a highly constrained time frame. The monitoring of drug distribution characteristics in granules (e.g. sieve fraction assay and blend uniformity) and content uniformity of active in developmental formulations offers very useful information concerning potential segregation and other process issues. The HPLC methods have been used for content uniformity and in-process sample analysis including sieve fraction assay to support formulation development. These methods are relatively time-consuming (25 min per sample analysis), and data turnaround time may not be optimal for fast paced early phase formulation development. As such, a fast and reliable method for formulation screening of the drug compound is highly desirable. The use of direct UV was attempted and the results indicated significant bias due to matrix interference. In this study, a rapid and reliable ICP-AES method was developed and validated for the determination of drug candidate **A** in its granule and tablet dosage form. The reliability and efficiency provided by this method has demonstrated its value in support of fast paced early stage formulation development.

2. Experimental

2.1. Materials

Drug compound **A** is available from Merck Research Laboratories (Rahway, NJ). Its tablet dosage forms and process granules including placebo tablets were manufactured by Pharmaceutical Research & Development of Merck Research Laboratories (West Point, PA). Concentrated hydrochloric, nitric, and sulfuric acids (optima trace metals grade) were obtained rom Fisher Scientific; 1 M hydrochloric, nitric and sulfuric acids were prepared by proper dilution of the corresponding concentrated acids. Deionized water was obtained from Fisher Scientific (Pittsburgh, PA) and used as received. Stock Calcium standard (1000 ppm in 2% HCl) was obtained from SPEXCertiPrep (Metuchen, NJ). Working Calcium standards were prepared by appropriate dilution of the stock standard solution with diluent (1 M HCl).

2.2. Instrumentation

A Perkin-Elmer Optima 3200 RL ICP-AES was used in this study with autosampler device (AS-90/91). The method conditions are summarized in Table 1. For comparison, a HPLC method was also used to analyze the samples where a Waters HPLC with a 717 autosampler, 600 controller, and 996 photodiode array detector were used.

2.3. Sample solution preparation (ICP-AES method)

One drug compound **A** tablet or approximately 200 mg granules were added in 60 ml 1 M HCl in a 100 ml volumetric flask. After mixing to ensure that the tablet and granules were disintegrated, extraction of calcium in drug compound **A** was conducted by heating at 90 °C for 2 h. The solution was allowed to cool to room temperature prior to diluting to QS, the resulting suspension was centrifuged and the clear supernatant was used for the analysis.

Table 1
ICP-AES method condition

Wavelength	317.933 nm
Sample flow rate	1.0 ml/min
Flush time	10 s
Replicates	3
Source equilibration	15 s
Plasma view	Radial
Viewing height	15 mm
Argon flow	15 l/min
Auxiliary flow	0.5 l/min
Nebulizer flow	0.8 l/min
RF Power	1300 W
Peak algorithm	Peak area

2.4. Standard solution preparation (ICP-AES method)

Standard solutions of 0.5, 1, 5, 10, 20, and 40 ppm were prepared from 1000 ppm calcium standard using 1 M HCl as the diluent and were used for the calibration and quantitation.

2.5. HPLC method conditions for content uniformity testing

An Inertsil ODS-3 column (4.6 mm i.d. $\times$ 250 mm) thermostated at 30 °C was eluted with a mobile phase consisting of acetonitrile/sodium phosphate buffer (0.025 M, pH 3) (70:30, v/v) at a flow rate of 1.3 ml/min. Drug compound **A** was determined by UV detection at 238 nm. The injection volume of 25 μl and run time was 13 min.

2.6. Sample solution preparation (HPLC method)

For uniformity testing, one tablet or equivalent amount of granule was placed into each of ten 250 ml volumetric flasks. The volume was diluted with a diluent consisting of acetonitrile/0.01 M sodium citrate buffer, pH 5.0 (50:50, v/v), and was stirred with a stirring bar for 3 h at fast speed until the tablets were dispersed in the solution. An aliquot was centrifuged and the supernatant was injected for analysis.

2.7. Standard solution preparation (HPLC method)

Approximately 27 mg of drug compound **A** reference standard was accurately weighed into a 250 ml volumetric flask and dissolved in the diluent consisting of acetontrile/0.01 M sodium citrate buffer, pH 5.0 (50:50, v/v) and mixed well for analysis.

3. Results and discussion

During the development of compound **A** tablet dosage form, HPLC method was initially used to support the sieve fraction assay, and tablet/granule uniformity testing. However, with a large number of samples for multiple formulations, relatively time-consuming HPLC was found not to be the method of choice when fast data turnaround was desired. Direct UV method was then attempted. However, under all circumstances, pronounced interferences from placebo matrices containing UV absorbing excipients (the maximum absorbance of **A** is 245 nm) were observed which often gave a bias of 2–10%. As the counter ion of drug compound **A**, calcium exhibits an atomic emission maximum at 317.9 nm as shown in Fig. 1. This provided us the opportunity to explore ICP-AES as the tool for quantitation of drug compound **A** in granules and tablet dosage forms.

3.1. Method development

Since the accurate determination of compound **A** depends upon its one to one stoichiometry ratio with calcium counter ion, that the proper selection of reagents and solvents used during ICP-AES method development is crucial to ensure that the residual calcium level (if any) would not have any significant interference of compound **A** quantitation. Calcium has distinct atomic emission at 317.9 nm, so this detection wavelength is selected for the method. Significant decrease in specificity and sensitivity were observed at other wavelengths. Three commonly used acids were evaluated as media for sample extraction and standard preparation. It was found that hydrochloric acid extracted the samples faster and more completely than sulfuric and nitric acids. Additionally, HCl is relatively non-corrosive and does not fume upon heating, as shown with sulfuric acid and nitric acid. Therefore, 1 M HCl was selected as the digestion medium. The impact of digestion time on drug recovery was studied. The results showed that after 2 h of digestion, the drug compound **A** can be totally recovered.

3.2. Method validation

3.2.1. Specificity

The sample diluent and placebo tablet solutions were injected per the method conditions. No significant interference ($< 2.0\%$) with the calcium signal of compound **A** was observed (Fig. 1). This

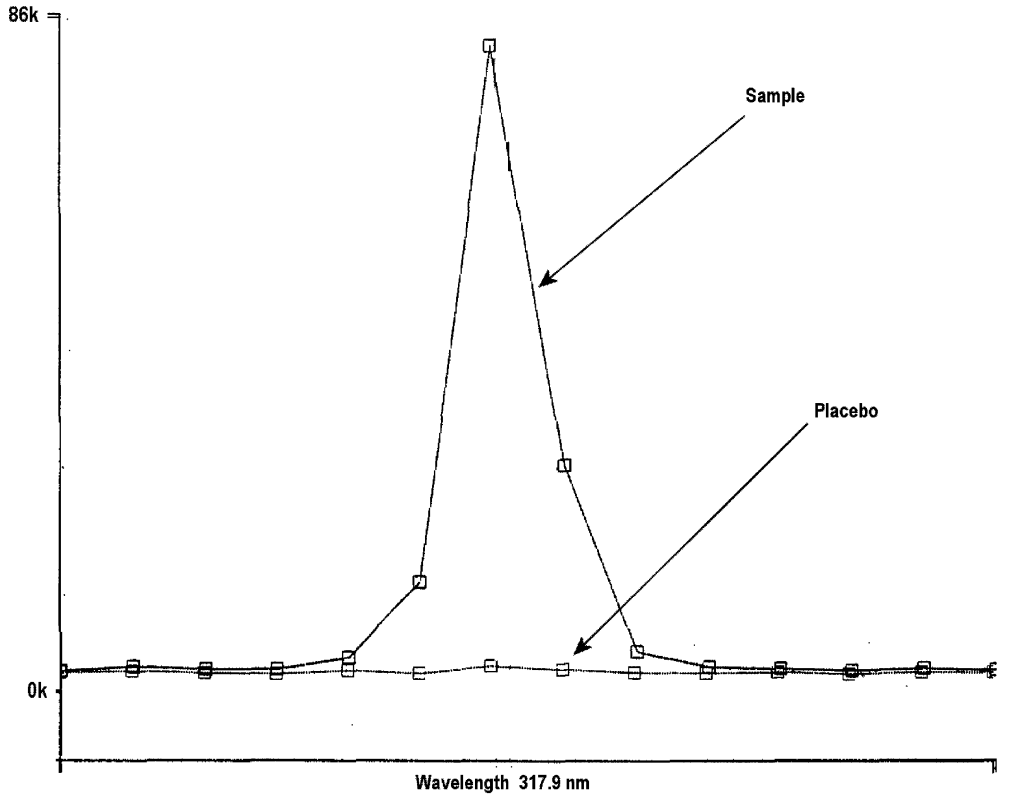

Fig. 1. ICP-AES spectra of drug compound **A** and placebo tablets solutions.

indicates that this method is specific and selective for the quantitation of calcium in drug compound **A**.

3.2.2. Sensitivity

The limit of detection (LOD) was estimated to be 0.1 ppm with a signal to noise ratio of greater than 3. The limit of quantitation (LOQ) was estimated to be 0.5 ppm with a signal to noise ratio greater than 10. The sensitivity of this method is adequate for the quantitation of calcium in samples (ca. 10 ppm).

3.2.3. Linearity

The linearity of drug compound **A** was established by preparing a series of placebo spiked with calcium standard solutions ranging from 0.5 to 40 ppm which corresponds to 5–400% of method concentration (10 ppm). The ICP-AES spectra were recorded using the diluent (1 M HCl) as a blank. All solutions were measured for emission at 317.9 nm. The resulting linearity of concentration vs. detector response was satisfactory with the coefficient (R^2) of 0.9999 (Fig. 2).

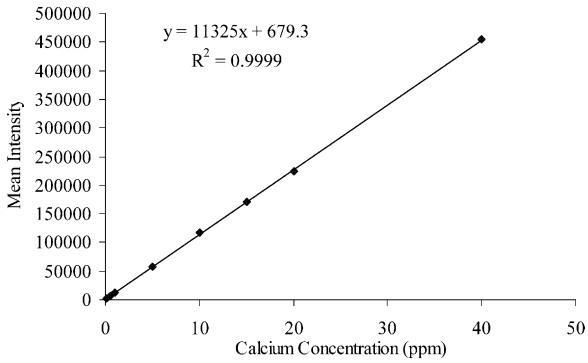

Fig. 2. Linearity of calcium standard solution with level of 10–400% of method concentration (10 ppm).

3.2.4. Accuracy

The accuracy of the method was determined by investigating the recovery of calcium at five levels ranging from 50 to 150% of the method concentration (10 ppm) from solution-spiked placebo tablets. The results are shown in Table 2, which indicate satisfactory recovery ranging from 96.7 to 101% with a mean of 99.6% (R.S.D. = 1.1%, N = 10).

3.2.5. Precision

The measurement precision was determined by performing ten replicate injections of standard solutions at the method concentration (10 ppm). The R.S.D. was found to be 0.44% by ICP-AES calcium measurement (Table 3). The method precision for the sample was determined by the analysis of ten drug compound **A** developmental tablets, the R.S.D. was 2.4% for these ten samples including tablet weight variation (Table 4).

3.3. Method application

This method has been applied to investigate the drug distribution characteristics in granule sieve fractions of two developmental formulations (1 and 2). For comparison, the same samples were analyzed by a validated HPLC method. As demonstrated in Fig. 3, the results generated by the ICP method and HPLC method correlate very well, and indicate more uniform drug distribution in developmental formulation 1. This method was also used for monitoring the content uniformity of drug compound **A** in two developmental tablet dosage forms. The results demonstrate excellent drug uniformity in both dosage forms, and are highly comparable to those results generated by the HPLC method (Fig. 4). The major advantage of ICP-AES spectrometer over HPLC is to quickly analyze large amount of samples without solution stability issues. In this study, analysis time for ICP-AES is less than 1 min per sample as

Table 2
Recovery of calcium from placebo tablets spiked in calcium standard solution

Level (%)	Mass added (mg)	Mass recovered (mg)	% Recovery
50	12.54	12.12	96.7
50	12.59	12.56	99.8
75	18.74	18.90	101
75	18.71	18.68	99.9
100	24.91	24.88	99.9
100	24.97	24.88	99.6
125	31.19	31.22	100
125	31.34	31.22	99.6
150	37.57	37.56	100
150	37.47	37.32	99.6
Average			99.6
R.S.D.			1.10%

Table 3
Measurement precision determined by ten replicate injection of a standard solution

Injection	Mean intensity
1	115 960
2	115 763
3	115 213
4	116 607
5	116 089
6	115 077
7	115 807
8	115 671
9	115 715
10	114 939
Average	115 926
R.S.D.	0.44

Table 4
Method precision determined by analyzing ten tablets of drug compound **A**

Tablet	Mass (mg)	Calcium (ppm)
1	131.00	10.6
2	129.49	11.0
3	129.42	11.1
4	128.76	10.9
5	128.29	11.3
6	129.27	10.7
7	128.09	10.8
8	128.73	11.1
9	129.03	11.1
10	130.12	10.6
Average		11.0
%R.S.D.		2.4

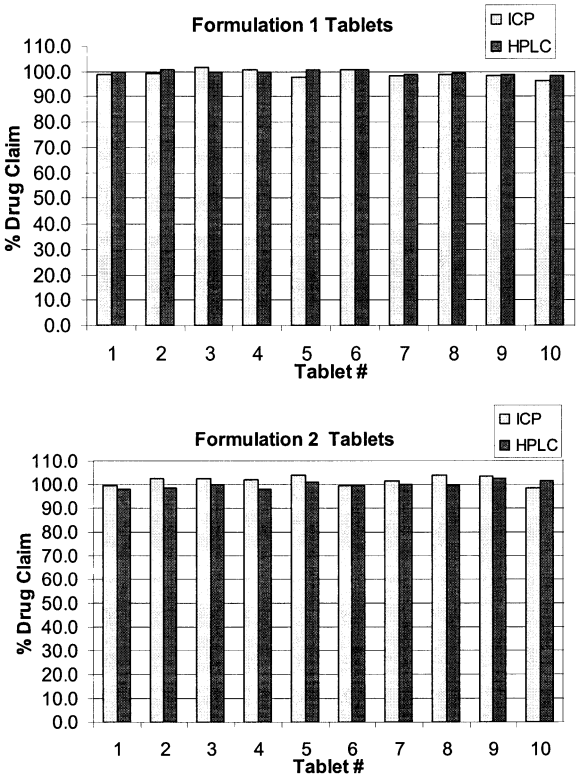

Fig. 3. Content uniformity of drug compound **A** in two developmental formulations as determined by ICP-AES and HPLC methods.

compared with 13 min per sample for the HPLC methods.

4. Conclusion

A simple, rapid and reliable method has been developed and used for the determination of content uniformity and distribution characteristics of an investigational drug **A** calcium salt in its granule and tablets dosage form. The results generated using ICP-AES are highly comparable with those from a validated HPLC method, but with significantly improved efficiency. This method offers rapid turnaround of analytical data and can be used as an excellent alternative to support fast paced formulation/process development.

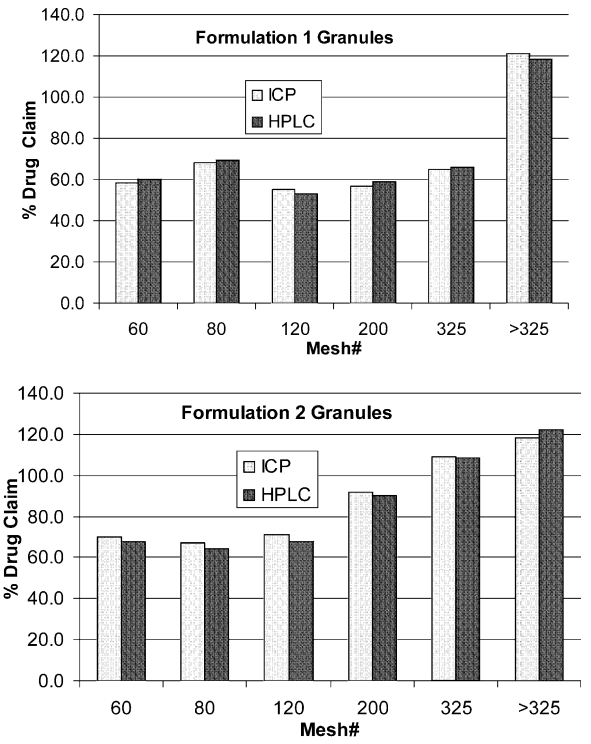

Fig. 4. Investigation of drug compound **A** distribution in granules from two developmental formulations by ICP-AES and HPLC methods.

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