

# Determination of Vincristine and Vinblastine in *Catharanthus Roses* Leaves by Liquid Chromatography Mass Spectrometry

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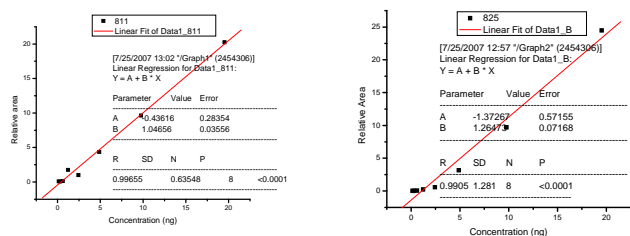
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## OVERVIEW

- ✓ An LC-MS method using TOF instrument has been developed for the determination of Vincristine and Vinblastine in *Catharanthus roses*
- ✓ Following solid-phase extraction of the sample, the lower limit of detection was 0.1 ng/ul and the linear range was 0.2-25 ng/ul
- ✓ Preliminary results are shown for *Catharanthus roses* samples

## INTRODUCTION

Vincristine and Vinblastine are alkaloids found in Rose Periwinkle (*C. Roseus*), a bushy perennial herb with a long list of traditional healing uses. Both Vincristine and Vinblastine are spindle inhibitors that demonstrate cell-specific inhibition of growth in the metaphase of mitosis. These compounds have application in cancer treatment and as such in large demand. Synthesis is costly, complicated and for vincristine unsuccessful. Extraction is complicated by low levels of analytes in the plant. This in turn generates a lot of interests to engineer Rose Periwinkle plants to produce significant amount of both alkaloids. The goal of the study is to establish a robust method to analyze the resulting plant materials to gauge its progress. To date, there are few published methods on quantifying the two drugs together using liquid chromatography. Mass spectrometry is a powerful tool for alkaloids analysis. In this study, we investigated the quantitative aspect of LC-MS for simultaneous determination of Vincristine and Vinblastine in plant leaf using a time of flight instrument. The established method could be applied to monitor the two drugs in plasma during variety of cancer treatment.



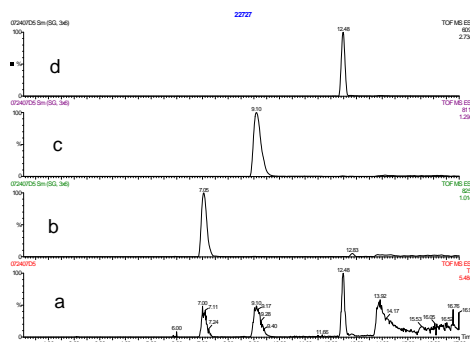
**Figure 2.** Determination of vincristine and vinblastine by LC-MS. (a, b) A standard curve revealed a linear detection response from 0.5-25 ng. Two fold dilution series were prepared in 2.4 ng standard solution. The peak area ratio of the two drugs versus internal standard was plotted against the corresponding concentration. The limit quantitation was 0.2 ng. Values are representative from one set of experiments. Similar result was obtained in a second set of experiments.

## EXPERIMENTAL

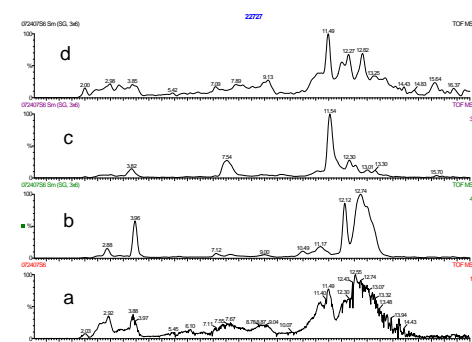
Intact plants were partitioned by leaf, stem, flower and root. Sample extraction was conducted as described (1). Briefly, sample leaves were collected, weighed and homogenized in a mortar with liquid N<sub>2</sub>. The leaf powder was transferred into a 50 ml centrifuge tube. The two drugs were extracted and enriched using liquid-liquid separation. Final extract was dissolved in 1 ml methanol. Vinblastine sulfate, Vincristine sulfate and Reserpine with > 99% purity were purchased from Sigma-Aldrich. To generate an external calibration curve, mixed standards of concentrations of 50, 25, 12.5, 6.25, 3, 1.5, 0.7, 0.1 ng/ul were prepared in 100% methanol. Reserpine was used as an internal standard. The samples were analyzed using a Waters Q-TOF2. Chromatographic separation was achieved on a C18 Beta basic 5μ 320 μm ID x 16 cm length (packed in house) in 12 min with a gradient elution employing 0.1% v/v formic acid +5% Acetonitrile+0.01%TFA as mobile phase A and MeOH/ACN (1:1) as mobile phase B. After data acquisition, an extracted ion chromatography was generated for vincristine, vinblastine and the internal standard at m/z of 825.5, 811.5, and 609.4, respectively. Peak integration was performed by MassLynx program (version 3.5, Waters) and the drug concentrations in leaves were quantified via linear regression analysis of the external calibration curve from the relative peak area.

## RESULTS

A Chromatogram of vincristine and vinblastine is shown below in **Figure 1**. Chromatographic separation of the two standards as well as the internal standard was achieved with acceptable resolution.



**Figure 1.** Separation and detection of vincristine, vinblastine and internal standard by LC-MS. (a) The total ion chromatography of standard mixture. The 3 compounds were completely resolved in LC monitored by positive mode ESI-MS. (b, c, d) Extracted ion chromatography of vincristine, vinblastine, and reserpine at m/z of 825.5, 811.5, and 609.4, respectively.



**Figure 3.** Assay of vincristine and vinblastine in leaves by LC-MS. (a) The total ion chromatography of leaves mixture. (b, c, d) Extracted ion chromatography of vincristine, vinblastine, and reserpine at m/z of 825.5, 811.5, and 609.4, respectively.

## CONCLUSIONS

TOF instrument was first used to quantify vincristine and vinblastine simultaneously in leaves. The sensitivity and linearity afforded by the method makes the studies of two drugs in plants possible. Ease of use, in terms of method development, was a notable feature of this study. The use of internal standard afforded only modest improvement in quantitation at higher concentrations, where the TOF detector began to saturate. We believe a MRM triple-quadrupole instrument might do better job here in terms of the dynamic range and linearity. Answer to this question will have to await the availability of a Q TRAP 4000 in the lab.

## Reference

- (1) Singh, D. V.; Maithy, A.; Verma, R. K.; Gupta, M. M.; Kumar, Sushil. Simultaneous determination of *Catharanthus* alkaloids using reversed-phase high-performance liquid chromatography. *Journal of Liquid Chromatography & Related Technologies* (2000), 23(4), 601-60