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# Determination of vincristine in infant plasma by liquid chromatography-atmospheric pressure chemical ionization-mass spectroscopy

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**Abstract**

An LC-MS method using APCI has been developed and validated for the determination of the anticancer drug vincristine in human plasma, using vinblastine as internal standard. Following solid-phase extraction (SPE) of the sample, the lower limit of quantitation (LLOQ) was 0.18 ng/ml, the lower limit of detection was 0.09 ng/ml, and the linear calibration range was 0.18–180 ng/ml. This method has been used to measure plasma concentrations of vincristine from 0.08 to 24 h post bolus in 29 infants as part of a pharmacokinetic study. Concentrations of vincristine at 24 h were 0.2–1.36 ng/ml.


*Keywords:* Vincristine; Vinblastine; Vinorelbine; Plasma; Analysis; Pediatric; Infant; LC-MS; APCI

## 1. Introduction

Vincristine, **1A**, is a vinca alkaloid isolated from *Catharanthus roseus*. It is used in the treatment of various childhood and adult malignancies, including acute lymphoblastic leukemia. Patients experience a wide range of tolerance to neurotoxic effects of the drug; a relationship between the area under the plasma concentration–time curve (AUC), and neurotoxicity has been described for adults [1].

Although vincristine is a component of established therapy for many childhood malignancies, few pharmacokinetic studies of vincristine in children have been published [2–6]. Of the children studied, only one pair of monozygous twins was infants [5]. A large part of this lack of pharmacokinetic data in infants is explained by limitations in determining very low concentrations of vincristine in plasma combined with limitations on the amount of plasma available. Vincristine is a weak chromophore with high quantitation limits by UV detection [7]. A radioimmunoassay [8] has been used to report a pharmacokinetic study in adults, but this assay is non-specific, as metabolites may cross react with the antibody [9–11]. Electrochemical detection has been used with off-line solid-phase extraction (SPE) [12] or on-line SPE [3,6] for pharmacokinetic studies in children and adults to achieve a lower limit of quantitation (LLOQ) of 0.5 ng/ml [6]. Electrochemical methods are difficult to optimize and troublesome to maintain due to their sensitivity toward the sample and chromatographic conditions [13–15]. The on-line SPE procedure has analyte recoveries of around 70%, the extraction column needs to be frequently replaced, and the peaks in the reported chromatogram have steeply sloping baselines. A chromatographic method for several vinca alkaloids using liquid–liquid extraction of plasma and employing APCI-MS has been reported [16]. The reported linear range for vincristine was 0.30–3.95 ng/ml; other vinca alkaloids reported had higher quantitation limits. The procedure used to extract the sample and the elution solvent used were different for each vinca alkaloid. This is not a critical limitation as patients will receive a single vinca alkaloid but a general extraction method is very desirable for potential transfer to clinical laboratories. No pharmacokinetic studies using this method have been reported.

Our objective was to develop a more sensitive assay to determine the pharmacokinetics of vincristine in infants. A sec-

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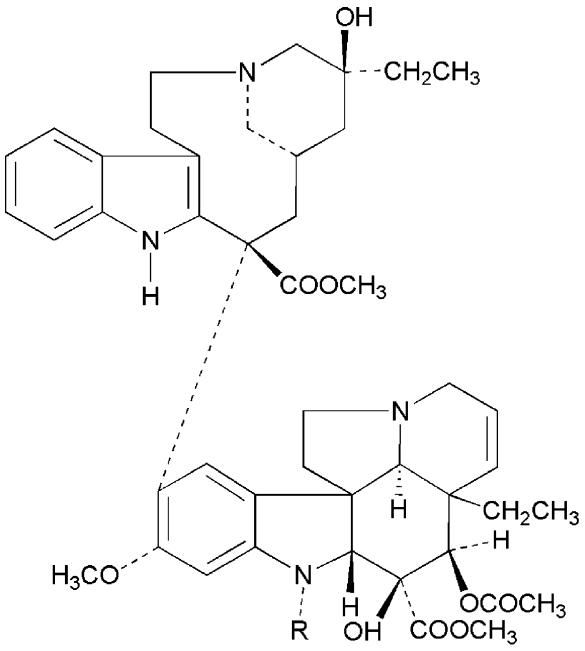
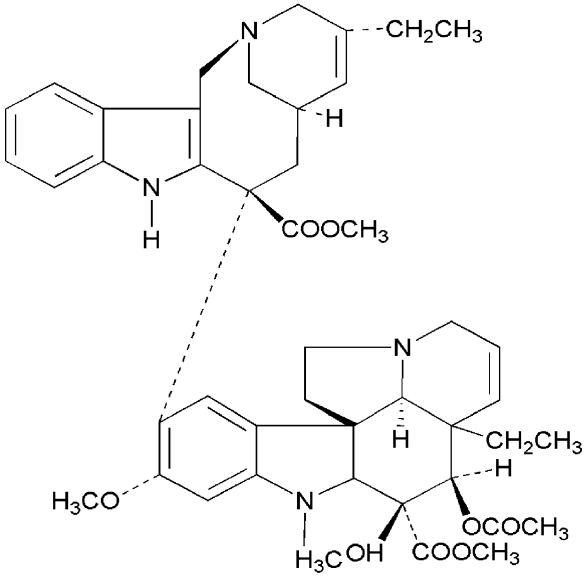



**1**




**2**

Fig. 1. Vincristine, **1A**, R = CHO; Vinblastine, **1B**, R = Me; and Vinorelbine, **2**.

ondary objective was to develop a robust and general method for vinca alkaloids that can be more easily replicated in other labs (Fig. 1). Infants receive a standard low-level dose of vincristine (0.05 mg/kg body weight) but can only supply a small amount of plasma (typically 0.5–1.0 ml) because of their limited blood supply volume. The number of samples in any study is small, therefore, sensitivity is much more important than speed or ease of automation. In this assay, vincristine is determined in plasma by LC-APCI-MS following sample cleanup and concentration via SPE.

## 2. Experimental

### 2.1. Chemicals and solutions

Vincristine sulfate injection was purchased from GensiaSicor Pharmaceuticals (Irvine, CA, USA) and was formulated to contain per ml 1 mg vincristine sulfate, 100 mg mannitol, acetic acid/sodium acetate at pH 3.5–5.5. Vinblastine sulfate injection was from APP (Schaumburg, IL, USA) and contained per ml 1 mg vinblastine sulfate, 9 mg sodium chloride, 0.09 ml benzyl alcohol at a pH from 3.5 to 5.0. Trifluoroacetic acid (TFA), *o*-phosphoric acid, acetic acid, HPLC-grade methanol and HPLC-grade acetonitrile were from Fisher Scientific. HPLC-grade water was from a MilliQ UV Plus® system from Millipore (Bedford, MA, USA). Donor plasma was obtained from the DeGowin Blood Center at University of Iowa Hospitals.

### 2.2. Blank plasma, plasma standards, and patient samples

To prepare plasma standards, vincristine sulfate injection was diluted with water to make an 8.94 μg/ml solution of vincristine. This standard was serially diluted with water to 894 ng/ml, 89.4 ng/ml, and 8.94 ng/ml of vincristine daily. Calibration standards and controls were prepared by adding between 10 and 100 μl of the appropriate working standard to sufficient human plasma to make 1.00 ml in a 2 ml tube. The working curve consisted of samples containing 0.18, 0.45, 0.9, 4.5, 8.9, 22.4, 44.7, 89.4, and 179 ng/ml of vincristine. Controls were 7.2 and 72 ng/ml vincristine. Internal standard solution was prepared by serially diluting vinblastine sulfate injection with water to 1000 ng/ml. Standards, blanks, null blanks, controls, and patient samples were prepared for SPE by adding phosphoric acid (50 μl) and internal standard (40 μl). All solutions were vortex-mixed and centrifuged for 1 min at 16,100 rcf. All samples were subjected to SPE prior to instrumental analysis.

### 2.3. Solid-phase extraction

Strata-X (30 mg/1 ml) SPE cartridges (Phenomenex Corporation, Torrance, CA) were used for sample preparation. A Cerex SPE processor from Varian (Palo Alto, CA) using nitrogen to modulate flow was used. All flow rates were approximately 1 ml/min. The cartridges were conditioned with 1 ml methanol followed by 1 ml water. After loading the sample, the cartridge was washed with 1 ml of 25% methanol/water and dried with nitrogen for 1 min. The analytes were eluted from the cartridge with 1 ml of 2% acetic acid/methanol. The eluate was collected in 13 mm × 100 mm glass tubes and solvent removed under flowing nitrogen at 35 °C. The residue was reconstituted in 100 μl of 54% (1:1 methanol: acetonitrile)/46% water and transferred to an autosampler vial for analysis.

The recovery of analyte from plasma was determined by spiking quadruplicate plasma samples with analyte at two concentrations and comparing the average peak area of the spiked sample to the average peak area of quadruplicate plasma samples with analyte added post extraction, followed by drying and reconstitution.

### 2.4. Instrumentation

The instrumentation system consisted of a Shimadzu LCMS-2010A mass spectrometer operating using APCI in positive detection mode controlled using LCMS solution (Version 2.04H3) software (Shimadzu, Columbia, MD, USA). The APCI source for this LCMS instrument provides maximum signal levels at typical lower flow rates used with 2 mm I.D. columns. The analytical column was a Phenomenex Polar-RP (4 μm, 80 Å, 2.0 mm × 250 mm) preceded by a Phenomenex Polar-RP SecurityGuard guard (2.0 mm × 4 mm) column. Separation conditions were sample temperature, 4 °C; column temperature, 23 (±3) °C; sample injection volume, 20 μl. The analysis was isocratic at 0.20 ml/min flow. Solvent A (46%) was 0.1% TFA/water; solvent B (54%) was 1:1 methanol/acetonitrile. The total run time for LC-MS analysis was 10 min.

The mass spectrometer was tuned using a polyethylene glycol solution following the manufacturer's protocol. The scan acquisition interval was 0.3 s, microscan 0.1 amu, the APCI temperature was 400 °C, the CDL temperature 250 °C, and the block temperature 200 °C. The probe voltage was 4.5 kV and the CDL voltage −15 V. Nitrogen flows: APCI, 2.5 l/min and drying gas, 0.02 MPa. Data was collected in selected ion monitoring (SIM) mode at 824.7 (vincristine) and 811.2 amu (vinblastine).

### 2.5. Calculations and precision

The vincristine concentrations were calculated from the peak-area ratio of vincristine to vinblastine for standards and samples. Patient samples were scaled to 1 ml when less than 1 ml of plasma was available. Previous work has demonstrated that SPE efficiency is independent of plasma volume [17] and a spot check with a spiked control demonstrated that this is also true for vincristine (data not shown). The linear least-squares equation was calculated with 1/concentration weighting. Correlation coefficients were 0.997 or better. A series of plasma controls spiked with vincristine at 0.18, 7.2, and 72 ng/ml were analyzed for accuracy and precision (Table 2). Ten samples of each concentration were analyzed to determine intraday reproducibility and the LLOQ. Fifteen samples of the two higher concentrations were prepared and analyzed in triplicate on 5 days to establish interday reproducibility. The LLOQ was determined according to FDA guidelines as a control value where the RSD is <20% and analyte signal is >5 times the blank matrix. The limit of detection was defined as an S/N of 3.

## 3. Results and discussion

Percent recovery of vincristine from plasma ranged from 90.3 to 104.7% (Table 1). Recovery was similar for vinblastine and vinorelbine, with percent recovery greater than 90% for all three vinca alkaloids (Table 1). Intraday relative standard deviations were <5% and interday relative standard deviations were <10% until near the LLOQ (Table 2). The limit of detection was determined to be 0.09 ng/ml.

Several studies of vincristine solutions have been performed. Most of the studies reported that vincristine solutions are stable for several days at 25 °C or less, are not sensitive to light, and store well in plastic containers [18–22]. It has also been reported that the storage and handling conditions of plasma samples containing vincristine is critical to the outcome of the analysis [23]. Spot checking of extracted samples in this study demonstrated stability in the autosampler over at least five days at 4 °C.

This assay was used for a pharmacokinetic study of vincristine in infants ranging from 4 days to 12 months old. Fig. 2a–c are illustrative chromatograms of **1A** and **1B** in an extracted standard, a patient sample 4 h post bolus, and a patient sample pre bolus. Fig. 3 shows the model fit plasma concentration–time curve obtained after bolus injection of vincristine (0.05 mg/kg). No analytical interferences from other drugs the patient were taking were observed. A total of 29 infants were studied and the data fit to a two-compartment model using ADAPT II software (Biomedical Simulation Resource, University of

Table 1
Analyte recovery from SPE processing, extracted in quadruplicate

| Analyte | 4 ng/ml | 40 ng/ml |
|---|---|---|
| Vincristine | 90.3% | 96.8% |
| Vinblastine | 104.7% | 95.1% |
| Vinorelbine | 99.4% | 99.3% |

Table 2
Precision and accuracy

| Concentration (ng/ml) | Accuracy (%) | RSD (%) |
|---|---|---|
| Intraday accuracy and precision ($n = 10$) | | |
| 0.18 | 110 | 19.6 |
| 7.2 | 97 | 4.2 |
| 72 | 108 | 1.8 |
| Interday accuracy and precision ($n = 15$), 3/day × 5 days | | |
| 7.2 | 96 | 9.2 |
| 72 | 103 | 6.3 |

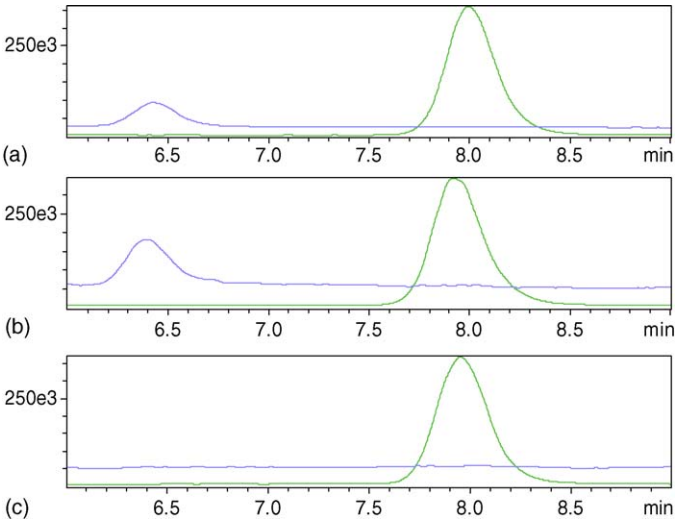



Fig. 2. Vincristine (6.43 min, 824.7 amu) scaled 10× for visibility with vinblastine (7.97 min, 811.2 amu, 40 ng/ml). *y*-Axis is intensity: (a) standard (0.45 ng/ml) extracted from plasma; (b) patient sample drawn 4 h post bolus; and (c) patient sample drawn immediately prior to bolus.

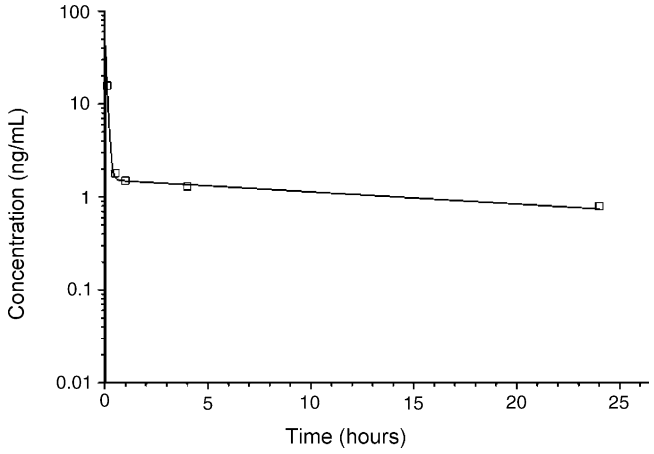



Fig. 3. Vincristine time course of patient 7, log concentration vs. time (h).

Table 3
Vincristine concentrations at various times

| Time (min) | Median (ng/ml) | Mean (ng/ml) (S.D.) | Range (ng/ml) |
|---|---|---|---|
| 5 | 18.3 | 26.6 (20.9) | 4.00–93.2 |
| 30 | 2.4 | 2.99 (2.02) | 0.93–11.1 |
| 60 | 1.78 | 1.82 (0.89) | 0.89–4.82 |
| 240 | 0.95 | 1.04 (0.33) | 0.48–1.95 |
| 1440 | 0.47 | 0.52 (0.24) | 0.20–1.36 |

Southern California, Los Angeles, CA). The interpatient variation in pharmacokinetic parameters seen previously was still present; however, the range in $t_{1/2\beta}$ and clearance values were less than seen previously. In fitting these parameters, at 440 min the (concentration)–time curve is still in transition between initial distribution of drug and elimination from the body. As a result, these values are mostly determined by the value obtained at 24 h. In adults and large children, some of the difficulties in fitting an appropriate curve can be lessened by collecting more data points after 4 h. For infants with a limited blood supply this approach is problematic.

A summary of concentrations at various times is found in Table 3. The high standard deviation at 5 min post bolus is likely due to discrepancies in actual versus theoretical sampling time. The importance of a very low LLOQ is apparent from the 24 h data.

## 4. Conclusion

Vincristine can be quantified at the 0.18 ng/ml level by LC-APCI-MS. The SPE procedure is general for the three vinca alkaloids tested, as is the chromatographic method. The sensitivity afforded by the method makes the clinical pharmacokinetic studies of vincristine in infants possible.

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