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Determination of vinca alkaloids in human plasma by liquid chromatography/atmospheric pressure chemical ionization mass spectrometry

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Abstract A sensitive assay was developed for the quantitation of vinblastine, desacetylvinblastine and vincristine using liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (LC-APCI-MS). Analyses were performed on an Ultrasphere C_{18} microbore column using ammonium acetate as mobile phase. The calibration curves were linear across the range of 0.51–4.00 ng/ml (0.63–4.93 n*M*) for vinblastine, 0.74–3.93 ng/ml (0.96–5.11 n*M*) for desacetylvinblastine and 0.30–3.95 ng/ml (0.36–4.79 n*M*) for vincristine. Vinca alkaloid concentrations were measured with an accuracy and precision within 11%. This assay could be implemented to determine the plasma concentrations for pharmacokinetic studies of vinblastine, desacetylvinblastine and vincristine in conjunction with clinical trials.

Key words Vinblastine · Desacetylvinblastine · Vincristine · Liquid chromatography-atmospheric pressure · Chemical ionization mass spectrometry

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Introduction

The vinca alkaloids are commonly administered for a wide variety of neoplasms including breast, bladder and lung cancers, lymphomas and leukemias. They exhibit high affinity for interfaces and surfaces, mandating careful assay design and validation. This, combined with the low concentrations of the vinca drugs in clinical samples, presents a significant analytical challenge. Several assays have been developed for the analyses of vinblastine, desacetylvinblastine and vincristine. Radioimmunoassays have been utilized [15], but they cannot differentiate between parent drug and the metabolites that may also bind to the antibody [3]. Vendrig et al. have developed a fluorescence method with a limit of quantitation of 0.5 ng/ml of vinblastine, but the assay cannot measure low concentrations of vincristine [22]. Other reported assays detect vinblastine and vincristine in the concentration ranges 1–100 [21] and 1–1000 ng/ml [20], and desacetylvinblastine in the ranges 1–100 [17] and 2.5–100 [21] ng/ml. A method to determine all three compounds at low nanomolar concentrations is needed.

Current methods for the determination of vincas use high-performance liquid chromatography (HPLC) with ultraviolet absorbance, fluorescence or electrochemical detection [3, 17, 20–22]. Liquid chromatography (LC), in combination with atmospheric pressure chemical ionization mass spectrometry (APCI-MS), provides a highly selective and sensitive method of detection. Our objective was to develop an LC-APCI-MS assay for the determination of vinblastine, desacetylvinblastine and vincristine in human plasma in a narrow concentration range (0.30–4.00 ng/ml). Quantitation was possible down to 0.51 ng/ml vinblastine, 0.74 ng/ml desacetylvinblastine and 0.30 ng/ml vincristine. Vinorelbine (5′-noranhydrovinblastine, Navelbine), a semisynthetic analog of vinblastine, was used as internal standard [7, 18, 19].

Materials and methods

Chemicals

Vinblastine sulfate (Velban) and desacetylvinblastine sulfate were obtained from Eli Lilly Co. (Indianapolis, Ind.). Vincristine, sodium salt, was purchased from Sigma Chemical Co. (St. Louis, Mo.). Vinorelbine tartrate (Navelbine), was kindly provided by Burroughs Wellcome Co. (Research Triangle Park, N.C.).

Methanol, chloroform, acetonitrile, ammonium acetate and potassium phosphate monobasic (all HPLC grade) were obtained from Fisher Scientific (Fair Lawn, N.J.). Absolute ethanol was purchased from McCormick Distilling Co. (Weston, Mass.). Deionized water was filtered using a Milli-Q water purification system (Millipore Corp., Bedford, Mass.).

Normal human plasma from healthy volunteers was obtained from the blood bank at the University of Chicago Hospitals (Chicago, Ill.).

Stock solutions and plasma sample preparation

Stock solutions of vinblastine, desacetylvinblastine and vincristine (40 μg/ml) were prepared in absolute ethanol. Subsequent dilutions were made in absolute ethanol and added to blank plasma. Drug concentrations in plasma were in the ranges 0.51–4.00 ng/ml for vinblastine, 0.74–3.93 ng/ml for desacetylvinblastine and 0.30–3.95 ng/ml for vincristine. Quality control samples within the specified concentration ranges were also prepared. Spiked plasma samples were aliquoted into amber borosilicate glass vials, and stored at −80 °C until analysis.

Extraction procedure

The extraction procedure described by Van Tellingen et al. [17] was used, with some modifications. Plasma samples (1.5 ml for the analysis of vinblastine and desacetylvinblastine, 2.0 ml for the assay of vincristine) were combined with the internal standard (400 ng/ml vinorelbine in ethanol), 5 ml 0.5 *M* potassium phosphate (pH 3 for the analysis of vinblastine and vincristine, pH 5 for the assay of desacetylvinblastine) and 5 ml chloroform. After vortexing vigorously for 30 s, the samples were centrifuged for 15 min at 2500 rpm and 4 °C. The aqueous layers were aliquoted into clean tubes and extracted one more time with chloroform. The organic phases were combined and reduced to dryness under nitrogen (37 °C). The residues were dissolved in 100 μl acetonitrile and injected onto the LC-APCI-MS column.

Equipment and chromatographic conditions

The LC-MS system consisted of a Hitachi Instruments (Tokyo, Japan) M-1000H LC-API mass spectrometer with an APCI interface and Hitachi LC modules (L-6200A pump and AS-4000 autosampler). Analyses were performed at room temperature on an Ultrasphere C_{18} microbore column (2 × 150 mm) packed with 5 μm material (Beckman Instruments, San Ramon, Calif.). The column was protected with a Novapak C_8 guard-pak (Millipore Corp., Bedford, Mass.). The LC effluent was nebulized (150 °C) in the APCI ion source. The electron multiplier and drift voltages were set at 2000 V and 85 V, respectively. The mass spectral data were obtained in the selective ion monitoring (SIM) mode. The instrument was calibrated using flow injection analysis (FIA). Before runs, the system performance was checked by injection of 1 ng vinblastine, desacetylvinblastine and vincristine onto the column.

The mobile phase for the determination of vinblastine consisted of 90% acetonitrile in 15 m*M* ammonium acetate (final concentration). Desacetylvinblastine was eluted using 90/10 15 m*M* ammonium acetate in acetonitrile/methanol. For the analysis of vincristine, 15 m*M* ammonium acetate in methanol was used as the eluent. The flow rates employed were 400 μl/min for the quantitation of vinblastine and vincristine, and 600 μl/min for the assay of desacetylvinblastine. The solvents were filtered using a nylon filter membrane, 0.45 μm, 47 mm (Micron Separations, Westboro, Mass.) and degassed under vacuum.

Determination of vinblastine, desacetylvinblastine and vincristine in human plasma

Calibration curves were generated by plotting the measured peak area (for vinblastine and desacetylvinblastine) and peak height (for vincristine) ratios (of drug over internal standard) versus the standard concentrations. A linear least-squares model, with a weight of $1/x^2$, was used. The concentrations of vinblastine, desacetylvinblastine and vincristine in plasma samples were determined from the calibration curves by interpolation.

Determination of recovery, precision and accuracy

The recovery from human plasma was determined from comparison of the peak areas (for vinblastine and desacetylvinblastine) and peak heights (for vincristine) of standards prepared in absolute ethanol with those of extracted plasma samples containing the same drug amount. Four replicates of quality control samples in plasma were processed to determine the intraday reproducibility. The interday reproducibility was calculated by performing duplicate analysis of standards in plasma over three consecutive days. The accuracy of the method was determined by expressing the mean interpolated concentration as a percentage of the spiked concentration. Ideally, the interpolated concentration should be 100% that of the spiked concentration. The precision of the assay was indicated as the percent coefficient of variation (%CV). This was calculated by dividing the standard deviation by the mean concentration value and multiplying this by 100. The smaller the %CV, the greater the precision.

Results

Typical selected ion mass chromatograms of extracted human plasma are shown in Figs. 1–3. The analytes were monitored as pseudomolecular ions $[M + H^+]$.

The assays were validated in terms of linearity, recovery, accuracy and precision. The observed peak area (for vinblastine and desacetylvinblastine) and peak height (for vincristine) ratios and drug concentrations in spiked human plasma were linear across the ranges 0.51–4.00 ng/ml for vinblastine, 0.74–3.93 ng/ml for desacetylvinblastine and 0.30–3.95 ng/ml for vincristine. The extraction efficiencies from human plasma spiked with 1.00 ng of vinblastine, desacetylvinblastine and vincristine were 87%, 74% and 80%, respectively. The accuracy of all assays ranged from 94 to 106% of the theoretical concentration throughout the standard curve (Tables 1–3). There was no relationship between concentration and accuracy ($r = 0.20$, $P = 0.708$

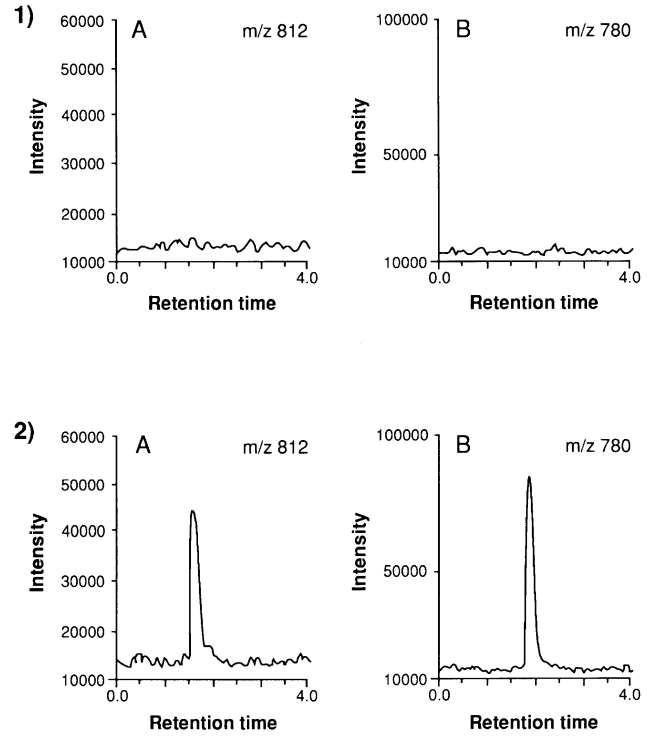

Fig. 1 Selected ion chromatograms obtained from extracted samples of (**1**) blank human plasma and (**2**) blank human plasma spiked with 1.01 ng/ml vinblastine and internal standard (*channel A* mass/charge (m/z) 812, vinblastine; *channel B* m/z 780, vinorelbine)

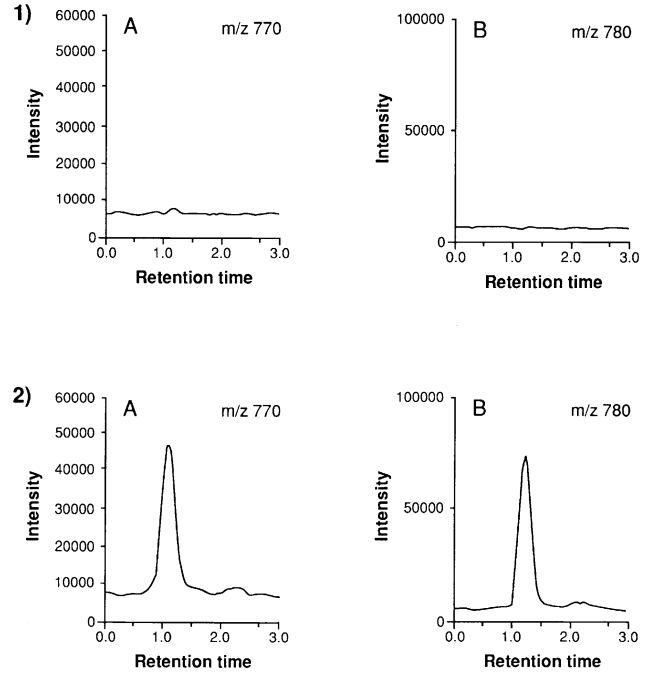

Fig. 2 Selected ion chromatograms obtained from extracted samples of (**1**) blank human plasma and (**2**) blank human plasma spiked with 1.02 ng/ml desacetylvinblastine and internal standard (*channel A* m/z 770, desacetylvinblastine; *channel B* m/z 780, vinorelbine)

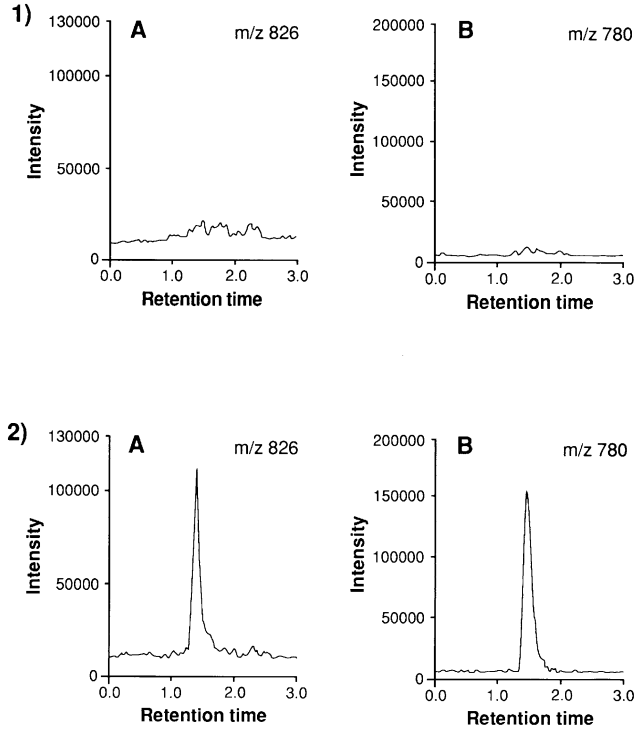

Fig. 3 Selected ion chromatograms obtained from extracted samples of (**1**) blank human plasma and (**2**) blank human plasma spiked with 1.75 ng/ml vincristine and internal standard (*channel A* m/z 826, vincristine; *channel B* m/z 780, vinorelbine)

Table 1 Inter- and intraassay accuracy and precision for the determination of vinblastine in human plasma by LC-APCI-MS

Theoretical concentration (ng/ml)	Mean found concentration (ng/ml)	Accuracy (% found/ theoretical)	Precision (%CV)
Interday			
0.51	0.54 ± 0.05	106	9.3
1.01	0.97 ± 0.07	96	7.2
1.73	1.63 ± 0.10	94	6.1
2.48	2.51 ± 0.10	101	4.0
3.26	3.29 ± 0.12	101	3.6
4.00	4.16 ± 0.22	104	5.3
Intraday			
1.01	1.01 ± 0.07	100	6.9
3.26	3.16 ± 0.27	97	8.5

for vinblastine; $r = -0.25$, $P = 0.628$ for desacetylvinblastine; $r = -0.44$, $P = 0.319$ for vincristine), and no difference in the accuracy for the vinca alkaloids assayed. The precision ranged from 2.5 to 10.5% (Tables 1–3). Precision was inversely correlated with vinblastine ($r = -0.81$, $P = 0.053$) and vincristine concentrations ($r = -0.89$, $P = 0.007$). However, no relationship ($r = -0.01$, $P = 0.989$) was seen for desacetylvinblastine.

Table 2 Inter- and intraassay accuracy and precision for the determination of desacetylvinblastine in human plasma by LC-APCI-MS

Theoretical concentration (ng/ml)	Mean found concentration (ng/ml)	Accuracy (% found/ theoretical)	Precision (%CV)
Interday			
0.74	0.77 ± 0.05	104	6.5
1.02	0.99 ± 0.06	97	6.1
1.73	1.81 ± 0.09	105	5.0
2.46	2.39 ± 0.20	97	8.4
3.22	3.23 ± 0.16	100	5.0
3.93	3.92 ± 0.25	100	6.4
Intraday			
1.01	1.02 ± 0.06	101	5.9
3.26	3.21 ± 0.08	98	2.5

Table 3 Inter- and intraassay accuracy and precision for the determination of vincristine in human plasma by LC-APCI-MS

Theoretical concentration (ng/ml)	Mean found concentration (ng/ml)	Accuracy (% found/ theoretical)	Precision (%CV)
Interday			
0.30	0.30 ± 0.03	100	9.9
0.50	0.51 ± 0.04	102	7.3
0.75	0.74 ± 0.07	99	9.4
1.00	1.05 ± 0.08	105	7.6
1.98	1.98 ± 0.09	100	4.7
2.98	2.89 ± 0.12	97	4.3
3.95	3.93 ± 0.16	99	4.0
Intraday			
0.40	0.38 ± 0.04	95	10.5
2.09	2.00 ± 0.11	96	5.4
3.72	3.76 ± 0.22	101	6.0

Discussion

Vinblastine and vincristine are often administered by continuous infusion for the treatment of various neoplastic diseases [5, 6, 11, 23, 24], resulting in plasma concentrations in the nanomolar range. Desacetylvinblastine is a metabolite of vinblastine found in humans [13]. Several assays for their quantification in body fluids have been developed [3, 16, 20–22], but more sensitive and specific methods are needed.

Combined LC-APCI-MS plays an important role in quantitative analysis [12], and has gained widespread recognition as a valuable analytical tool [9]. It has been successfully employed for the determination of drug levels in biological fluids [1, 4, 8, 9], and offers the multiple benefits of high sensitivity, selectivity and specificity. The extraction procedure and mobile phase composition were optimized for each individual analyte to eliminate interferences and maximize recovery and sensitivity. The vinca recoveries are dependent on the pH of the phosphate buffer [17]. For vinblastine and vincristine, the optimum pH was 3, whereas for desacetylvinblastine, a pH of 5 worked better. Previous methods for the analysis of vinca alkaloids have used phosphate buffers as mobile phase additives [3, 14, 17, 20, 21], but the interfaces employed in MS analysis require the use of volatile buffers. In our assay we used ammonium acetate, a very common volatile buffer [2] and ionizing additive. It aided in the elution of the vinca alkaloids from the column, and prevented the clogging of the interface and the formation of deposits in the MS source [16]. Small changes in the mobile phase composition altered the ionization efficiency for each analyte. For vincristine, the use of acetonitrile had a quenching effect on the ion signal when compared to methanol. A mobile phase of 15 m*M* ammonium acetate in methanol was chosen for its analysis. For vinblastine, the opposite effect was observed and 15 m*M* ammonium acetate in acetonitrile was used. A combination of ammonium acetate-acetonitrile-methanol yielded the highest ion intensity for desacetylvinblastine. Since cancer patients are not simultaneously treated with both vinblastine and vincristine, it is not inconvenient that separate runs are needed for their analyses. For the assay of all vinca alkaloids a microbore column was used, which reduced the dilution of the samples and allowed the detection of the analytes as sharp peaks. This in turn increased the signal to noise ratio resulting in better sensitivity [10].

The data presented in this study demonstrate an LC-APCI-MS assay developed to quantitate vinblastine in human plasma in the concentration range 0.51–4.00 ng/ml, desacetylvinblastine in the range 0.74–3.93 ng/ml and vincristine in the range 0.30–3.95 ng/ml. This assay will be employed to determine the plasma concentrations for pharmacokinetic studies of vinblastine, desacetylvinblastine and vincristine in conjunction with clinical trials. This approach might also be useful for the determination of other vinca alkaloids in human plasma, such as vinorelbine, vindesine and vinzolidine.

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