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Simultaneous quantification of vinblastine and desacetylvinblastine concentrations in canine plasma and urine samples using LC–APCI–MS/MS

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ABSTRACT

A highly sensitive and specific liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry (LC/APCI–MS/MS) method has been developed and validated for simultaneous quantification of vinblastine and its metabolite, desacetylvinblastine, in canine plasma and urine samples. Plasma and urine samples were processed by a solid phase extraction procedure. The optimal chromatographic behavior of these analytes was achieved on pentafluorophenyl (PFP) propyl analytical column (5 μm, 50 × 2.1 mm) under isocratic elution of 0.75 mL/min with a mobile phase of 5 mM ammonium acetate and methanol. The samples were analyzed in positive ion, multiple reaction monitoring mode. The calibration curves were linear over 0.125–2 ng/mL (lower calibration curve); 2–100 ng/mL (higher calibration curve) and 0.125–5 ng/mL for vinblastine and desacetylvinblastine in plasma, and over 1–2000 ng/mL and 0.5–100 ng/mL for vinblastine and desacetylvinblastine in urine samples, respectively. The limits of quantitation of vinblastine and desacetylvinblastine were 0.125 ng/mL in both matrices. The intra and interday accuracy was above 89% and precision below 8.6% for both analytes in both matrices. The developed method was successfully applied to ongoing *in vivo* vinblastine pharmacokinetic studies in dogs.

1. Introduction

Vinblastine (vincaleukoblastine; VLB) is a vinca alkaloid isolated from dicotyledonous Madagascar periwinkle plant, *Catharanthus roseus* (family: Apocyanaceae). Vinblastine was the first vinca alkaloid isolated and has been used for the treatment of soft tissue tumors both in human and veterinary medicine as a single agent or in combination with other anticancer drugs. 4-O-desacetylvinblastine (DVLB) is a metabolite of vinblastine that is itself considered to be a potent and active anticancer xenobiotic [1]. Vinca alkaloids work by binding to beta-tubulin and preventing the formation of microtubules during mitosis, thereby arresting cell division [2–4]. Vinca alkaloids were also shown to inhibit angiogenesis [5]. In order to increase the therapeutic utility or decrease the potential for toxic side effects, semi-synthetic vinca alkaloids, such as vinflunine, vindesine sulfate and vinorelbine (VRB) were developed. Although all of these vinca alkaloids share common features in structure and affinity to microtubules, there is variability in their therapeutic efficiency, therapeutic indications, and toxicities [6]. Currently, toxicity adjusted dosing is the most accepted method of dose adjustment for vinblastine administration in dogs [7,8].

A variety of methods, including radioimmunoassay and high performance liquid chromatography (HPLC) coupled with ultraviolet (UV), fluorescence, or electrochemical detection have been used to quantify vinblastine concentrations in biological matrices [9–12]. A reversed phase – HPLC/fluorescence method was successfully developed in this laboratory for the analysis of *vinca* alkaloids with a maximal sensitivity of 1 ng/mL. Use of the HPLC/fluorescence method to assess preliminary pharmacokinetic samples in canine plasma indicated the need for an assay with even greater sensitivity, with quantitation of vinblastine plasma concentrations well below 1 ng/mL. As species specific differences in blood and urine constituents can result in matrix effects and differences in analyte recovery, assay methods must be validated in the species of interest. In addition, the natural variability found between dog breeds can result in considerable intersubject variability, necessitating the use of an assay with a wide working range in analyte concentrations. To the best of the authors' knowledge, there are no published reports on highly sensitive and specific analytical methods to quantify the concentrations of vinblastine and desacetylvinblastine in canine plasma and urine. The literature indicated successful liquid chromatography tandem mass spectrometry (LC/MS/MS) methods with the required sensitivity [13], so this laboratory began pursuing an LC/MS/MS method for the quantification of

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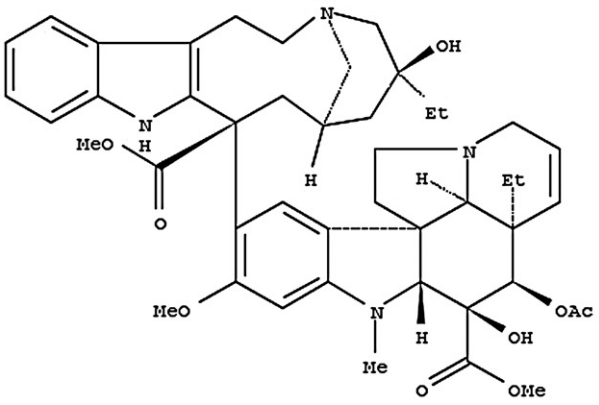
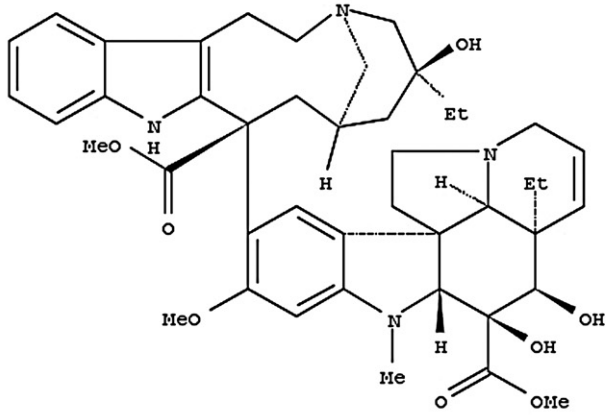
Vinblastine (811)



4-O-Desacetylvinblastine (769)



Vinorelbine (779)

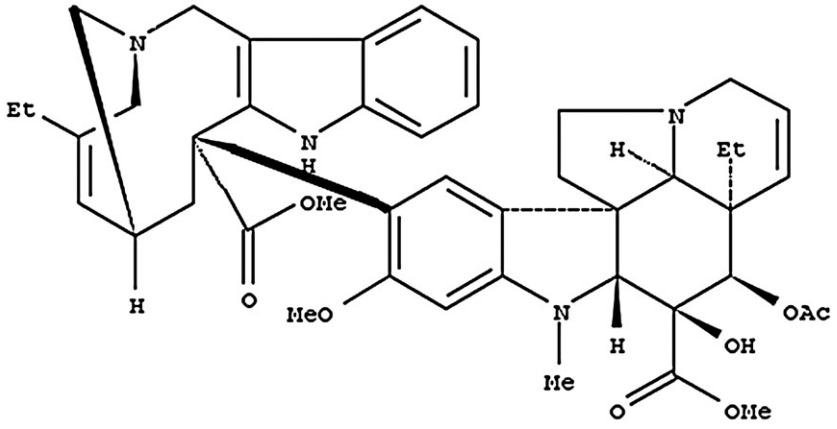


Fig. 1. Chemical structures of vinblastine, desacetylvinblastine, and vinorelbine with molecular weights in parenthesis.

vinblastine by modifying several previously published methods for the analysis of vinca alkaloids [13–15]. In LC/MS/MS, monitoring precursor drug mass and product ion fragment mass gives high specificity in the quantitation of a drug. Sensitivity of the LC/MS/MS is also higher than that of other analytical methods. The use of LC/MS/MS is becoming more prevalent in drug metabolism and *in vitro* screening of new chemical entities (NCEs) in drug development process because of its high sensitivity and specificity [16].