DIONEX

Application Brief 122

Separation of Etoposide Using an Acclaim Phenyl-1 Column

INTRODUCTION

Etoposide, a semisynthetic derivative of podophyllotoxin, is used in treating patients with a variety of malignant tumors.[1] Its analysis by reversed-phase high-performance liquid chromatography (HPLC) has been published by the United States Pharmacopeia (USP) and Chinese Pharmacopoeia (CP).[2,3]

The USP related compounds method uses a 50 min gradient on a column containing packing L11. The USP column packing L11 is defined as phenyl groups chemically bonded to porous silica particles 1.5 to 10 µm in diameter. The particle size requirement in the USP etoposide monograph for the related compounds method is less than 5 µm in diameter. The resolution (R_s) between etoposide and propylparaben required in the related compounds test should be not less than 1.1. The CP method is similar to the USP method but adds a requirement that the retention time (t_R) of etoposide be approximately 25 min.

The work shown here describes a separation of etoposide using an Acclaim® Phenyl-1 column. The Phenyl-1 column is based on covalent modification of high-purity, spherical, porous silica particles (3 µm), with a specially designed silane ligand-bearing proprietary alkyl aromatic functionality.[4]

Figure 1A shows chromatograms of etoposide and propylparaben following the USP method. The R_s between etoposide and propylparaben is 8.7, much better than required in the USP method; and the t_R of etoposide is close to 25 min, which meets the requirement in the CP method.

Figure 1B shows a faster and simpler method for the separation of etoposide and propylparaben using the Phenyl-1 column with isocratic elution. The separation of etoposide is completed within 7 min with excellent resolution (R_s = 5.2) between etoposide and propylparaben.

EQUIPMENT

Dionex UltiMate® 3000 RSLC system including:

- HPG 3400RS pump
- WPS 3000RS autosampler
- TCC-3000RS thermostatted column compartment
- DAD-3000RS UV-vis detector

Chromeleon® Chromatography Data System (CDS) software version 6.80 SR9

REFERENCES

1. Kato, Y.; Mawatari, H.; Nishimura, S.I.; Sakura, N.; Ueda, K. Determination of Etoposide Serum Concentrations in Small Pediatric Samples by an Improved Method of Reversed-Phase High-Performance Liquid Chromatography. *Acta Med. Okayama* **2003,** *57* (1), 21–24.
2. The United States Pharmacopeia, USP34–NF29, 2788.
3. Chinese Pharmacopoeia (Vol. 2), 2010, 472.
4. Dionex Corporation, *Acclaim Phenyl-1 Unique Reversed-Phase Column with High Aromatic Selectivity.* Sunnyvale, CA. http://www.dionex.com/en-us/products/columns/lc/reversed-phase/acclaim-phenyl/lp-87227.html (accessed Jan 6, 2011).

Column:	Acclaim Phenyl-1 (4.6 × 150 mm, 3 µm)
Column Temp.:	30 °C
Mobile Phase Panel A:	Solution 1) CH_3CN/20 mM NaAc (pH 4.0), 20:80 (v/v) Solution 2) CH_3CN/20 mM NaAc (pH 4.0), 40:60 (v/v)

Gradient Time	*Solution 1 (%)*	*Solution 2 (%)*
0	100	0
0-15	100	0
15-30	100→40	0→60
30-40	40	60
40-42	40→0	60→100
42-45	0	400
45-47	0→100	100→0
47-50	100	0

Mobile Phase Panel B:	Solution 2) CH_3CN/20 mM NaAc (pH 4.0), 40:60 (v/v)
Flow Rate:	1.5 mL/min
Injection Volume:	10 µL
Detection:	UV, 254 nm
Chromatograms:	a) Blank (solution 1) b) Etoposide (100 µg/mL) c) Mix of etoposide (90 µg/mL) and propylparaben (8 µg/mL))

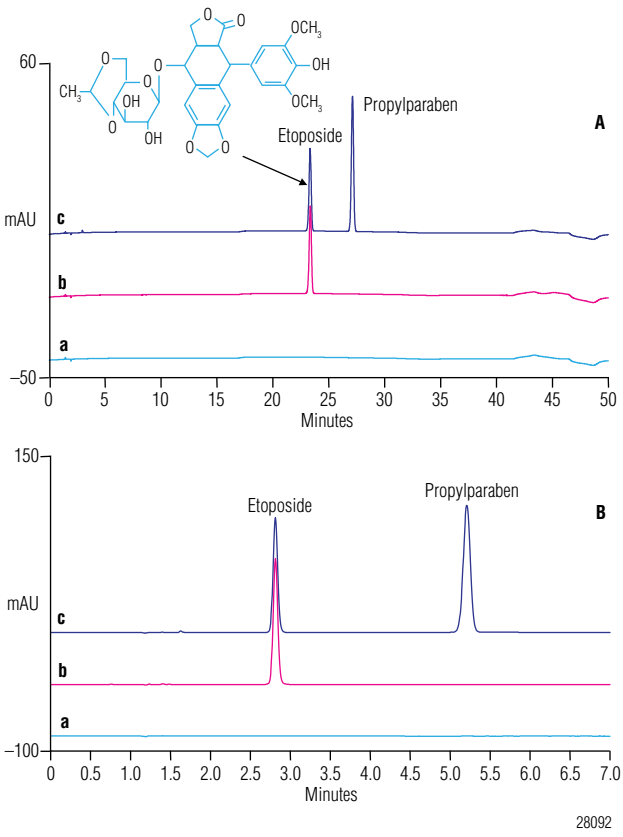

Figure 1. Chromatograms of etoposide following (A) the USP method and (B) isocratic method, using the Acclaim Phenyl-1 column.

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