

## **Application Note**

747010H

### Analysis of Antiflatuents by High Performance Liquid Chromatography with Evaporative Light Scattering Detection

#### Introduction

Medicines contain various active ingredients and for analyzing such ingredients simultaneously, gradient elution method is useful, requiring high sensitive detection method. Therefore, for analyzing medicines in gradient mode it is quite useful to use Evaporative Light Scattering Detector (ELSD), because it can detect almost all the compounds except volatile components with high sensitively and stable baseline.

In this report, some ingredients contained in Antiflatulent were analyzed by using ELSD and PDA detector, such as Stearic Acid that is used as lubricants for forming tablets, Ursodeoxycholic Acid that is effective to improve digestion and absorption, Acrinol that is used as disinfectant and Berberine that has antibacterial, anti-inflammatory and gastric mucosal protection effects.

Keyword: Antiflatuent, C18 column, PDA detector, ELSD

Conditions

#### **Experimental**

<u>Equipment</u>		Conditions	
Pump:	PU-2089	Column:	CrestPak C18S (4.6 mmID x 150 mmL, 5 μm)
Autosampler:	AS-2057	Eluent A:	10 mM Ammonium acetate in Acetonitrile
Column oven:	CO-2060	Eluent B:	10 mM Ammonium acetate
Detector:	ELS-2040	Gradient condition:	$(A/B)$ , $0 \min (5/95) \rightarrow 15 \min (70/30) \rightarrow 15.05 \min (95/5) \rightarrow$
			$20 \min (95/5) \rightarrow 20.05 \min (5/95)$ 1 cycle: 35 min
		Flow rate:	1.0 mL/min
		Column temp.:	40°C
		ELSD condition:	Nebulizer temp.: 30°C
			Evaporator temp.; 60°C
			Gas flow rate; 1.0 SLM
		PDA wavelength:	200-650 nm

 $10 \mu L$ 

Stearic acid, Acrinol, Ursodeoxycholic acid, Berberine

Fig.1 shows structural formula of ingredients contained in antiflatuent.

Injection volume:

Standard sample:

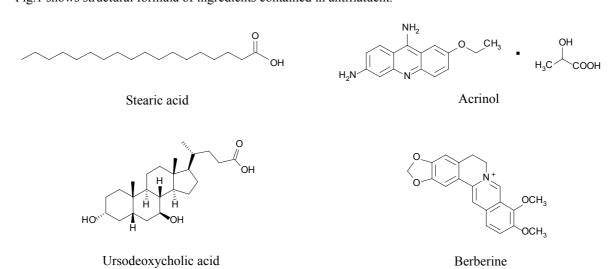


Fig. 1. Structural formula ingredients contained in antiflatuent

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#### Result

Fig.2 shows chromatogram of standard mixture and Fig. 3 shows chromatogram of antiflatuent. Upper data of each figures is chromatogram detected by ELSD and lower data, detected by PDA detector (Wavelength: 220 nm) respectively. By PDA detector, only two ingredients were detected, while all four ingredients were detect clearly by using ELSD.

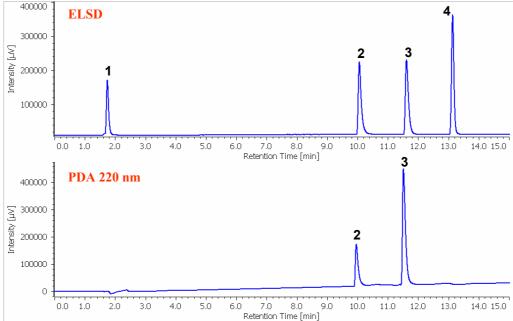


Fig. 2. Chromatogram of Standard mixture

1: Stearic acid (0.5 mg/mL), 2: Acrinol (0.1 mg/mL), 3: Berberine (0.1 mg/mL), 4: Ursodeoxycholic acid (0.1 mg/mL)

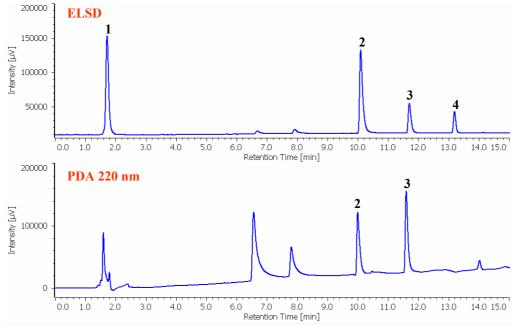


Fig. 3. Chromatogram of Antiflatuent

1: Stearic acid, 2: Acrinol, 3: Berberine, 4: Ursodeoxycholic acid

Preparation: A 1.0 mg/mL solution of Antiflatuent smashed into powder was prepared by methanol and filtrated through the  $0.45~\mu m$  membrane filter.