Analysis of 7-ethoxycoumarin in a Biological Matrix Using the Agilent 500 Ion Trap LC/MS

Application Note

Clinical Research

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Abstract

A highly specific and robust LC/MS/MS method for analysis of 7-ethoxycoumarin (7-EC) in a biological matrix (plasma) is described. Results showed calibration in-matrix from 0.01–10 µM with r(2) > 0.998, and LLOD in-matrix is 2 pg on column.

Introduction

7-ethoxycoumarin (7-EC) (Figure 1) is a commonly used probe for metabolism studies. It has been used to determine whether isolated hepatocytes can be successfully used to predict in vivo clearance values [1]. It has also been used in developing rainbow trout liver perfusion techniques for studies on xenobiotic biotransformation [2]. 7-ethoxycoumarin O-de-ethylation has been used widely as a marker for assessing substrate specificities of cytochromes P450 (P450) in liver microsomes of mammals [3]. This application note describes a highly specific and robust LC/MS/MS method for analysis of 7-ethoxycoumarin in a biological matrix.

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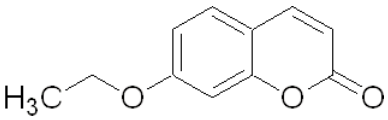

Figure 1. Structure of 7-ethoxycoumarin.

Instrumentation

The following instrumentation was used for this study:

- Agilent 500 Ion Trap LC/MS with ESI source
- Agilent Prostar 420 AutoSampler
- Agilent 212-LC Binary Solvent Delivery Modules

Materials and Reagents

7-ethoxycoumarin (7-EC) (CAS number 31005-02-4) and reserpine (CAS number 50-55-5) were obtained from Sigma Aldrich, St. Louis, MO.

CD-1 plasma obtained from Bioreclamation Inc., Hicksville, NY.

All other chemicals were reagent grade or HPLC grade.

Sample Preparation

Plasma was spiked with 7-EC using serial dilution to obtain nine levels of standard from 10 µM to 0.01 µM. Thirty five microliters of each standard was pipetted and mixed with 35 µL of the internal standard (I.S.), reserpine (0.5 µM). Sample preparation was performed using protein precipitation with three times the volume of acetonitrile followed by 0.45 µm filtration using the Agilent Captiva filtration system (p/n A5967045K).

Mobile phase A was added to the filtrate in order to lower the percent organic to match the initial mobile phase conditions.

HPLC conditions

Column:	Pursuit XRs C18, 150 × 2 mm id, 5 µm (p/n A6000150X020)			
Mobile phase A:	0.1% formic acid in water			
Mobile phase B:	0.1% formic acid in acetonitrile			
Injection volume:	20 µL			
LC program:	Time (min:sec)	%A	%B	Flow (µL/min)
	0:00	60.0	40.0	200
	5:00	15.0	85.0	200
	5:30	15.0	85.0	200
	5:42	60.0	40.0	200
	7:00	60.0	40.0	200

API and MS parameters

Drying gas:	25 psi at 400 °C
Nebulizing gas:	25 psi
Needle:	5000 V
Spray shield:	600 V
Enhanced scan mode:	5000 Da/sec

Analyte	Precursor ion *(m/z)*	Product ion *(m/z)*	Product ion range *(m/z)*	Capillary (V)	RF loading (%)	CID (v)
7-ethoxy-coumarin	191.0	163.0	162–164	74	72	0.85
Reserpine	609.3	397.3	397–398	74	72	2.41

Results and Discussion

The 500 Ion Trap was optimized for 7-ethoxycoumarin and reserpine. Optimized values of scan parameters were used in the method. Duplicate standard curves were prepared and injected.

A nine-level calibration curve was constructed spanning four orders of magnitude from 0.01 µM to 10.0 µM with the following results (Figures 2 and 3):

r^2 = 0.9998

LLOQ = 0.01 µM

ULOQ = 10 µM

Using MS/MS, baseline noise was extremely low, typically < 110 counts for 7-EC and < 20 counts for reserpine. There was no interference from the blank.

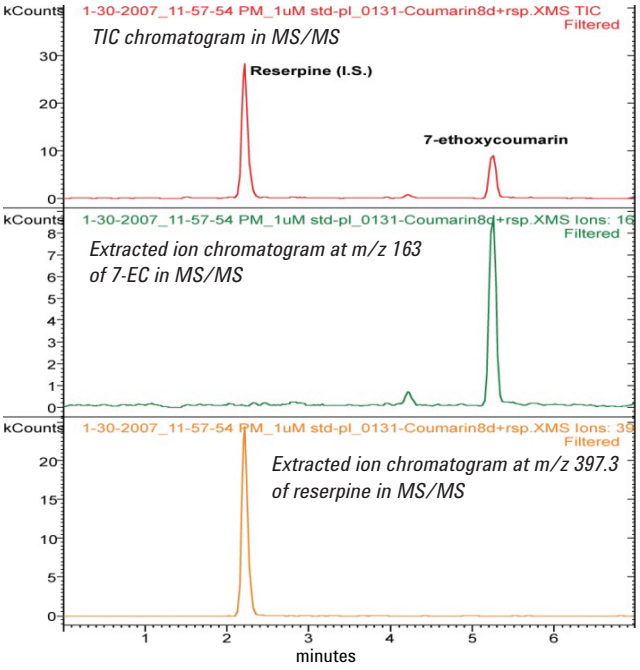

Figure 2. Typical chromatogram of 7-ethoxycoumarin and reserpine (I.S.) from plasma at 1 µM level.

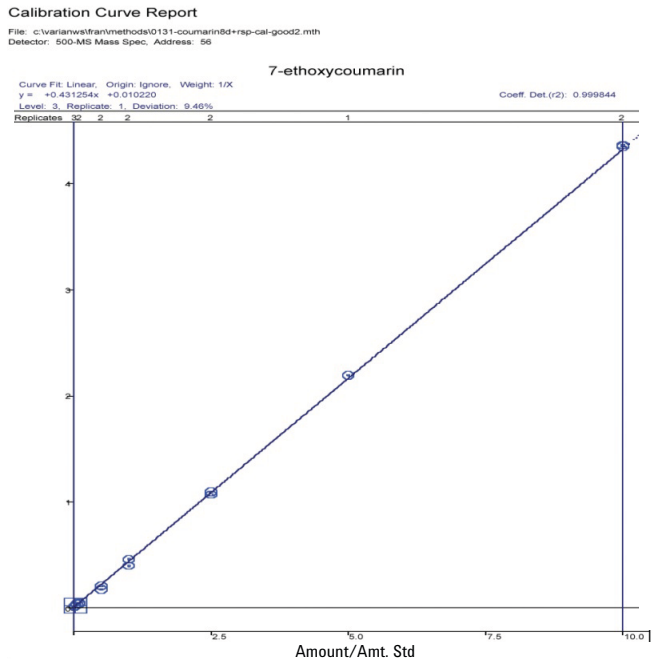

Figure 3. Duplicate standard curves of 7-ethoxycoumarin extracted from plasma. Excellent linearity ranging from 0.01 µM to 10.0 µM. r^2 = 0.9998.

A standard similarly prepared at 0.002 µM established the LLOD to be 2 pg on-column, with S/N = 6 (Figure 4).

The reproducibility of multiple injections (6) of the same vial of 0.1 µM standard was determined:

RSD of peak area	7-EC	5.30%
	I.S.	5.10%

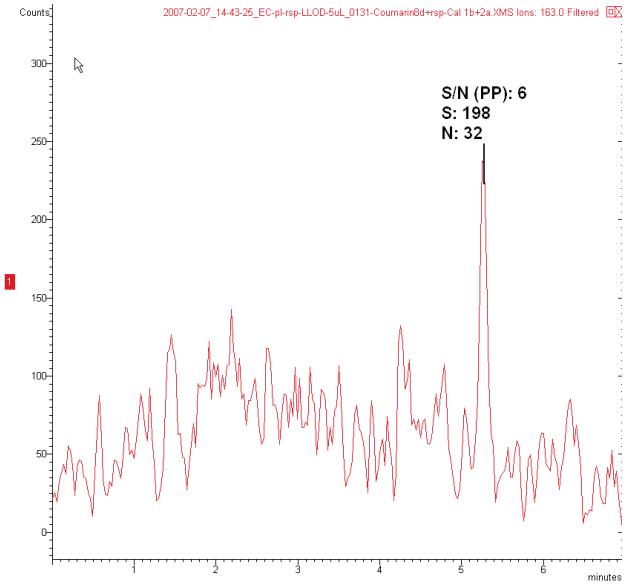

Figure 4. LLOD determined to be 2 pg of 7-ethoxycoumarin on-column, with S/N = 6.

Conclusion

The Agilent 500 Ion Trap LC/MS provides excellent performance for the analysis of 7-EC in plasma:

- Calibration in-matrix from 0.01 – 10 µM has $r^2 > 0.998$
- LLOD in-matrix is 2 pg on-column

References

1. Carlile et al., *Drug Metabolism and Disposition* 26 (1998) 216.
2. Andersson et al., *Drug Metabolism and Disposition* 11(1983) 494.
3. Yamazaki et al., *Biochemical Pharmacology* 51 (1996) 313.

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Printed in the USA
May 18, 2012
SI-0932

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