Simultaneous Extraction and Quantitation of Fentanyl and Norfentanyl from Primate Plasma with LC/MS Detection

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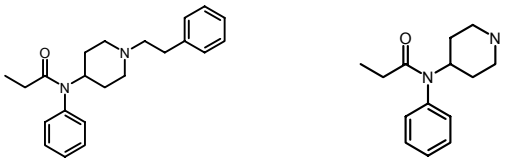
The quantitation of fentanyl and its desalkyl metabolite, norfentanyl, in blood plasma using LC/MS detection has not been previously described. This application note reports the successful detection and quantitation of these basic drugs using a Zirchrom®-PBD column. Mass spectroscopy detection was performed using ESI in the positive mode. The LOQ for fentanyl was 25 pg/ml and norfentanyl was 50 pg/ml.



Fentanyl Norfentanyl

Figure 1. Structures of Fentanyl and Norfentanyl

Introduction

Transmucosal fentanyl is an analgesic agent used in the control of cancer pain in humans and as a presurgical sedative for children [1,2]. This method was developed by the Zoological Pharmacology Laboratory, College of Veterinary Medicine, Kansas State University (Manhattan, Kansas, USA) to support a pharmacokinetic/pharmacodynamic study of transmucosal fentanyl as a preanesthetic in chimpanzees, orangutans, and gorillas [3]. Along with obtaining data on fentanyl plasma concentrations, it was also desirable to have information on the metabolism of fentanyl in these three species of primates.

There are currently no published extraction and detection procedures that quantitate both fentanyl and norfentanyl from plasma using LC/MS. Fentanyl in plasma has been quantitated using LC [4] and radioimmunoassay [2]. Furthermore, the lowest published level of detection for fentanyl in plasma was 100 pg/ml. The assay reported here allowed quantitation to 25 pg/ml for fentanyl and 50 pg/ml for norfentanyl. The liquid-liquid extraction used toluene as the organic phase [5].

Experimental

A mixture of fentanyl and norfentanyl was separated at room temperature using a ZirChrom®-PBD column and an LCQ_{DUO} LC/MS system manufactured by ThermoFinnigan (San Jose, CA) using an ESI source with positive ionization. The separation conditions were as follows:

Column:	ZirChrom®-PBD, 50 mm x 2.1 mm i.d., 3 micron (Part Number: ZR03-0521)
Mobile Phase:	45/55 (v/v) acetonitrile/10 mM ammonium acetate, 0.1 mM citrate (pH 4.4)
Temperature:	Uncontrolled
Flow Rate:	0.3 ml/min.
Injection:	50 µl
Detection:	LC/MS/MS

This method results in a sensitive and accurate assay that allows for the quantitation of both fentanyl and norfentanyl from primate plasma. The liquid-liquid extraction combined with the sensitivity of MS detection has allowed lower quantitation concentrations of both compounds than previously reported [5].

Table 1: Chromatographic Results for Fentanyl and Norfentanyl

Compound	Retention Time	k'
Fentanyl	2.24	2.11
Norfentanyl	4.86	5.75

This method can be tailored to your specific application needs. ZirChrom method developers can help to optimize and transfer this method to your site. Please contact ZirChrom technical support at 1-866-STABLE-1 or support@zirchrom.com for details.

ZirChrom phases offer unique selectivity, high efficiency, and excellent chemical and thermal stability.

Acknowledgements

D.E. Koch, R. Isaza, J.W. Carpenter, and R.P. Hunter, Zoological Pharmacology Laboratory, College of Veterinary Medicine, Kansas State University (Manhattan, Kansas, USA)

References

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