ALMAC

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CASE STUDY

An HPLC-MS/MS Method for the Determination of Oxycodone, Noroxycodone & Oxymorphone in Human Plasma

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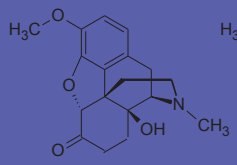
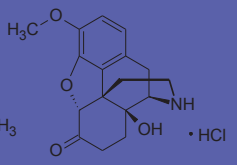
BACKGROUND

Oxycodone, an opiod analgesic that closely resembles morphine, is often used for the control of pain in cancer and organ transplant patients. An HPLC-MS/MS method was developed and validated according to current industrial guidelines for the determination of oxycodone and its active metabolites noroxycodone and oxymorphone, in human plasma in the range of 0.200-100 ng/mL. The deuterated species of each compound interest was used as its respective internal standards. The analytes was extracted from plasma using HCX solid phase extraction cartridges.

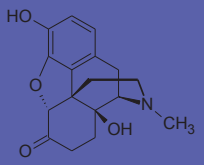
Figure 1: Structures of the Oxycodone, Noroxycodone and Oxymorphone



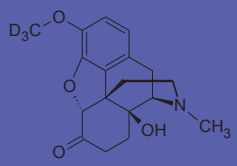
Oxycodone
(C18H21NO4 MW 315)



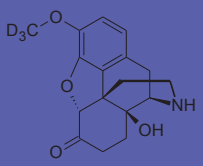
Noroxycodone
(C17H19NO4 MW 301)



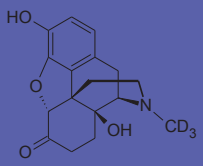
Oxymorphone
(C17H19NO4 MW 301)



Oxycodone-D3
(C18H18D3NO4 MW 318)



Noroxycodone-D3
(C17H16D3NO4 MW 304)



Oxymorphone-D3
(C17H16D3NO4 MW 304)

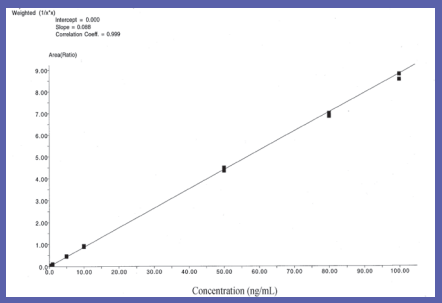
Method

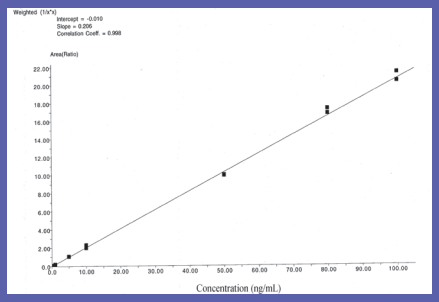
Calibration curves and QC samples were prepared in blank human plasma.

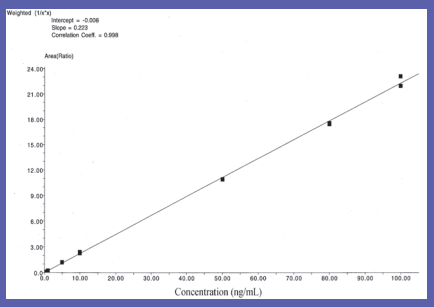
Assay Type:	HPLC-MS/MS
Analysis Volume:	1.00 mL
Analytes:	Oxycodone (Internal Standard Oxycodone-D3)
	Noroxycodone (Internal Standard Noroxycodone-D3)
	Oxymorphone (Internal Standard Oxymorphone-D3)
Sample Extraction:	HCX solid phase extraction after addition of internal standards
Mass Spectrometer:	Sciex API 365 or API 3000
Calibration Range:	0.200-100 ng/mL

Results

Standard curves were produced by linear regression (weighted) using the peak area ratios of analyte to each respective deuterated species used as internal standard. During validation the correlation coefficient (r) was observed ≥0.995 for all analytes (n=14 (7 concentrations in duplicate).

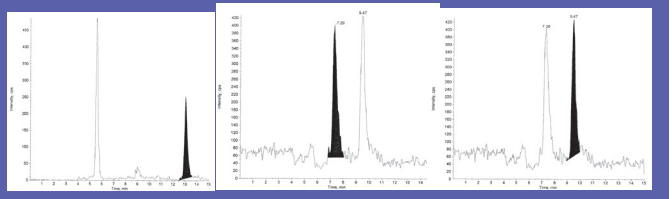




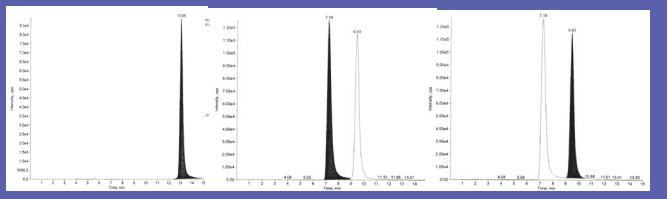


HPLC-MS/MS Chromatograms for Analytes in Human Plasma

Linear range of .200-100 ng/mL for each analyte
LLOQ (0.200 ng/mL)



High Standard (100 ng/mL)



Oxycodone | Noroxycodone | Oxymorphone

Accuracy and Precision

Intra- and inter-run accuracy and precision were established by analysing six replicate QC samples, at six different levels, i n four primary runs. Back-calculated versus nominal concentrations were used to determine accuracy (%RE). Precision was expressed by the standard deviation and %CV. Intra-run %RE (n=6) was within ±11.5% (±15.9% at LLOQ) and the %CV ≤14.9% (≤12.8& at LLOQ) overall all three analytes.

Analyte	Plasma nominal concentration (ng/mL)	Inter-run (N=24) %RE	%CV
Oxycodone	(LLOQ) 0.200	1.3	12
	0.500	-0.4	8.7
	2.00	-2.6	4.1
	40.0	3.8	4.1
	75.0	-2.3	4.7
Noroxycodone	(LLOQ) 0.200	10.5	9.5
	0.500	-4.0	10.3
	2.00	0.3	5.8
	40.0	-3.3	4.5
	75.0	-6.1	3.1
Oxymorphone	(LLOQ) 0.200	0.5	10.4
	0.500	-5.1	8.3
	2.00	-7.1	4.3
	40.0	-3.4	4.3
	75.0	-6.6	4.1

Extraction Efficiency From Spike Samples

Extraction Efficiency was calculated by comparing the peak area from extracted QC sample analysis versus mean peak area of blank samples spiked after extraction at 3 different levels (0.500, 40.0 and 75.0 ng/mL of oxycodone, oxymorphone and noroxycodone and their respective internal standards (5.00 ng/mL). Percent extraction efficiency ranged:

Oxycodone	82.9-95.4%
Oxymorphone	79.5-87.9%
Noroxycodone	70.9083.2%
3 Internal Standards	77.2-88.9%

Dilution Analysis

Aliquots of plasma spiked at 400ng/mL for oxycodone, oxymorphone and noroxycodone were diluted prior to analysis by a factor of 5- or 20-fold with blank human plasma. The mean recovery obtained for diluted samples were within ±6.5% RE of the nominal concentration. The precision was ≤4.8% CV.

Stability

The stability of oxycodone, oxymorphone and noroxycodone were tested by analysing three replicas of fortified plasma samples at two concentrations, 0.500 and 75.0 ng/mL.Stability was established:

- Two freeze/thaw cycles in plasma.
- 48 hours in the autosampler at ambient, ~20°C post extract.
- 24 hours at bench top (ambient), in plasma.
- 21 weeks at -20°C and -70°C, in plasma.

Stability was also established for the analytes in reagent:

- 16 weeks at 4°C.

Conclusion

A sensitive, simultaneous and specific HPLC-MS/MS method for the determination of oxycodone and two of its active metabolites, oxymorphone and noroxycodone has been validated in human plasma. The results obtained during the validation of the method proved the suitability of the HPLC-MS/MS assay for the determination of oxycodone, oxymorphone and noroxycodone in human plasma. Stability of the analytes under conditions of analysis were established.

References

Morphine or cancer pain? Heiskanen T.E. et al.; Acta Oncol 2000; 39(8):941-947.

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