CIBTech Journal of Pharmaceutical Sciences ISSN: 2319–3891 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/cjps.htm
2015 Vol.4 (2) April-June, pp.8-14/Hu and Shi
Research Article

DETERMINATION OF OXYCODONE CONTENT AND RELATED SUBSTANCES IN OXYCODONE AND ACETAMINOPHEN CAPSULE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

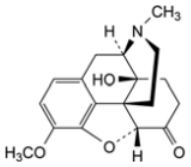
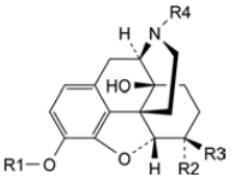
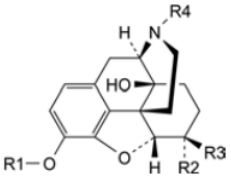
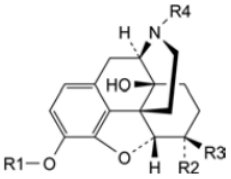
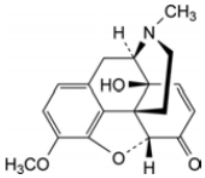
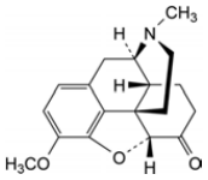
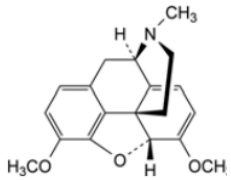
***Liandong Hu[1,2] and Yanjing Shi[1,2]**
[1]School of Pharmaceutical Sciences, Hebei University, PR China
[2]Key Laboratory of Pharmaceutical Quality Control of Hebei Province, Baoding, PR China
**Author for Correspondence*

ABSTRACT
A selective, rapid and sensitive high-performance liquid chromatography (HPLC) method has been developed for the simultaneous determination of oxycodone and related substances in oxycodone and acetaminophen capsule. An isocratic elution was performed to determine content of oxycodone on a Welchrom C_{18} column (5μm, 250 ×4.6 mm) for the separation, with 7mM potassium dihydrogen phosphate aqueous solution (containing 0.1% phosphoric acid, 0.1% n-nonylamine, with potassium hydroxide solution (1 → 2), the pH was adjusted to 4.9 ± 0.1) and methanol (9:1) as the mobile phase at the flow rate of 1 mL/min.The detection wavelength for oxycodone was 214 nm. The accuracy of this method, measured by the recovery of oxycodone was above 99% at three spiking levels. The linear regression analysis data for the calibration.plots showed good linear relationship at the concentration range. Gradient elution was used in order to assay the contents of related substances at the detection wavelength of 230nm. All the parameters of recovery study were within the limits. Both of the two methods showed good linearity for oxycodone and related substances, respectively. The main component oxycodone was well separated from other ingredients and degradation products. Both of the two methods were capcable to confirm the contents of corresponding substances. This method is fast, simple, and can be used for determination of oxycodone and related substances in this oxycodone and acetaminophen preparation.

Keywords: *Oxycodone, Related Substances, HPLC, Isocratic Elution, Gradient Elution*

INTRODUCTION
Oxycodone ($C_{18}H_{21}NO_4$) is a semi-synthetic opioid with an agonist activity on mu, kappa and delta receptors. Equivalence with regard to morphine is 1:2.
Its effect commences one hour after administration and lasts for 1-2 h in the controlled-release formulation.
Plasma halflife is 3–5 h (half that of morphine) and stable plasma levels are reached within 24 h (2–7 days for morphine) (Davis and Wilcock, 2001; Leow *et al.*, 1995).
Oral bioavailability ranges from 60 to 87% (Poyhia *et al.*, 1993; Davis *et al.*, 2003). Oxycodone metabolism is more predictable than that of morphine, and therefore titration is easier. Side effects are those common to opioids, but it causes somewhat less nausea, hallucinations and pruritus than morphine (Maddocks *et al.*, 1996; Davis and Tailcock, 2001; Ripamonti and Bruera, 1997).
Above all, oxycodone is an effective analgesic; it has been used in many ways, such as in the terminal stages of cancer, in the postoperative phase and so on. Also, it has been used in the form of tablets and capsules.
There are several process associated with the manufacture and synthetic routes of oxycodone drug substance may introduce related substances.
Different related impurities are observed in the whole manufacturing processes. Qualitation and quantification of oxycodone along with its related substances is required for accurate and precise procudure potentially present in formulated pharmaceuticals. The structure and chemical names of oxycodone and its related substances are presented in Table 1.

Table 1: Chemical names and structures for oxycodone hydrochloride and its related substances

	Chemical names	Structures
(oxycodone hydrochloride),active ingredient	4,5α-Epoxy-14-hydroxy-3-methoxy-17-methylmormorphinan-6-one-hydrochloride	
Oxymorphone, impurity A	R1=H,R2=R3=O,R4=CH_3:4,5α-epoxy-3,14-dihydroxy-17-methylmormorphinan-6-one	
7,8-dihydro-14-hydroxycodeine,impurity B	R1=R4=CH_3,R2=OH,R3=H:4,5α-epoxy-3-methoxy-17-methylmormorphinan-6α,14-diol	
noroxycodone, impurity C	R1=CH_3,R2+R3=O,R4=H:4,5α-epoxy-14-hydroxy-3-methoxymormorphinan-6-one,	
14-hydroxycodeinone, impurity D	7,8-didehydro-4,5α-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one	
Hydrocodone, impurity E	4,5α-epoxy-3-methoxy-17-methylmorphinan-6-one	
Thebain, impurity F	6,7,8,14-tetradehydr-4,5α-epoxy-3-6-dimethoxy-17-methylmorphinan	

In this study, we develop two methods to separate oxycodone and its related substances in oxycodone and acetaminophen capsule: an isocratic HPLC method and a gradient method for the determination of oxycodone and its related substances respectively.

MATERIALS AND METHODS

2.1 Reagents and Apparatus

HPLC grade acetonitrile and methanol was purchased from Yuwang industrial co., LTD, Shandong, China. Analytical grade n-nonylamine, phosphoric acid and potassium hydroxide were obtained from Alfa Aesar a Johnson Matthey, USA, Beijing Chemical Works. Tianjin Chemical Reagent, respectively. Oxycodone hydrochloride reference standard was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The oxycodone and paracetamol tablets were purchased from Mallinckrodt Inc. (USA). Double distilled water was used during the entire HPLC procedure.

The HPLC analysis system consisted of a Empower 2 liquid chromatogram and Waters 2489 detector and the chromatographic column was a Welchrom C18 (5μm , 250 ×4.6mm).

2.2 Preparations and Chromatography

2.2.1 Analytical Samples

Sample stock solution: Weigh the contents of 10 capsules. Mix and transfer an accurately weighed portion of the powder, equivalent to about 5.0 mg of oxycodone hydrochloride, to a 50ml volumetric flask, add 10ml methanol and added with solvent mixture (methanol: water=4:1) to volume. The resulting solution was passed through a 0.45μm membrane filter.

Sample solution: Dilute the 1ml stock solution with solvent mixture to a 10ml volumetric flask.

2.2.2 Standard Solutions

An oxycodone stock solution containing oxycodone at a concentration of 100.0 μg/mL allowed accurate and precise quantitation of oxycodone, then the stock solution was diluted with solvent to 10μg/mL.

2.2.3 Chromatographic Conditions

For oxycodone： The mobile phase composed of 7mM potassium dihydrogen phosphate aqueous solution (containing 0.1% phosphoric acid, 0.1% n-nonylamine, with potassium hydroxide solution (1 → 2), the pH was adjusted to 4.9 ± 0.1) and methanol (9:1) as the mobile phase at the flow rate of 1.0 mL/min.The selected detection wavelength was 214nm. The injection volume was 20μL.

For related substances: Mobile phase A consisted of a mixture of 830mL heptanesulfonic acid sodium salt monohydrate solution at the concentration of 1.1 mg/mL(adjusted the pH to 2.0 with the mixture of the same amount of phosphoric acid and water) and 70mL acetonitrile along with 100ml methanol. Mobile phase B consisted of a mixture of 600 mL heptanesulfonic acid sodium salt monohydrate solution at the concentration of 1.1 mg/mL(adjusted the pH to 2.0 with the mixture of the same amount of phosphoric acid and water) and 150mL acetonitrile along with 250ml methanol.The mobile phase at the flow rate of 1.5 mL/min.The selected detection wavelength was 230 nm. 20 μL of samples were injected into the HPLC for each analysis.The temperature of the column was 40℃.The mobile phase gradient applied is performed in Table 2.

Table 2: Gradient profile used for the separation oxycodone and the related substances

Gradient time (min)	%A mobile phase	%B mobile phase
0	100	0
60	50	50
62	100	0
70	100	0

2.3 Validation Studies

The developed method was validated for system suitability, specificity, linearity, precision, accuracy, LOD and LOQ, following ICH recommendations.

2.3.1 System Suitability

The system was deemed to be suitable for use if the following acceptance standard were satisfied. The tailing factor for oxycodone was not more than 2.0.The relative standard deviation (RSD) of the peak area

responses for oxycodone from other related substances was not more than 2.0%. The resolution between the oxycodone and other related substances was not less than 1.5.

2.3.2 Calibration Curves

Linearity of the method was evaluated at five concentrations level. All of the solutions consisted of oxycodone and its related substances. The resulted peak areas were inputted into a Microsoft Excel spread-sheet to plot the calibration curves. And the correlation coefficients were calculated.

2.3.3 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Solutions of oxycodone and its related substances were prepared by dissolving known amounts of the prepared stock solutions to different assay concentrations in the solvents. Each of the prepared solutions was prepared in duplicate and chromatographed.The signal-to-noise ratios for oxycodone and the related substances were calculated. The limit of detection (LOD) was measured as the lowest amount of the solutions that could be detected to produce a significant response; the signal-to-noise was about 3:1. The quantification limit for each solution was determined based on the value of signal-to-noise, which was about 10:1.

2.3.4 Accuracy

Each sample was prepared by weighing oxydocone and its related substances and dissolving with solvents to three levels.To assess accuracy, each of the samples were prepared in triplicate, and the peak areas were used to calculated mean and %R.S.D. values.

2.3.5 Precision and Specificity

The instrumental precision was investigated by analyzing six consecutive injections of low, middle and high concentration standard solutions on the same day and on 6 different days. Specificity is the ability of the method that we mentioned above was capable of measuring the peak response of analyses in presence of all potential components (eg. impurities, degradation products, adjuvant *et al.*,).

2.3.6 Stability

During the initial stage of the method development and the whole stage of experiment, the impurity stock solution was prepared by dissolving all the analytes in the solvent.The stability of sample solutions for oxycodone tablets was tested after storing for 24h at the condition of room temperature. The stability results obtained at the end of the experiment were compared to the initial concentrations to evaluate the stability of oxycodone solutions.

RESULTS AND DISCUSSION

Various mobile phases and columns were used to arrive at a method that achieved an optimal separation for all the components.

The chromatographic method described here separates the related substances of oxycodone. In order to seek a perfect method than can assay the determination of oxycodone and its related substances preferably, various mobile phases, temperature and columns were tested. The chromatographic method described here can separate oxycodone from its related substances. Related substance D and E cannot separate with each other, so the contents of D and E were assayed together.

System Suitability

The key system suitability parameters, including theoretical plates and asymmetry factors for chromatographic peak of oxycodone were calculated as European Pharmacopoeia described. The tailing factor for oxycodone was not more than 1.3.The relative standard deviation (RSD) of the peak area responses for oxycodone from other related substances was not more than 2.0%. The resolution was greater than 3.0 or higher.

Validation Results

Calibration Curves

3.2.1. Accuracy and recovery for oxycodone and related substances

The results of the recovery studies show that the method is accurate for the determination of oxycodone. The individual oxycodone recoveries for placebo samples spiked at 2.5–10 µg/ml of label claim ranged from 99.7 to 100.6%.

Table 3: Accuracy recovery for oxycodone and related substances

	Recovery (%)	Mean(%)	RSD%	Mean(%)
Oxycodone	99.4		0.3	99.9
	99.8	99.7		
	100.0			
	99.7		0.7	
	99.7	99.3		
	98.5			
	102.5		1.7	
	100.1	100.6		
	99.3			
Impurities A	100.1	0.5	0.5	99.6
	99.1			
	100.4			
	98.1	0.8		
	99.6			
	100.0			
	100.0	0.2		
	99.7			
	99.7			
Impurities B	100.1	0.1	0.6	99.7
	100.0			
	100.0			
	98.4	0.8		
	98.6			
	100.2			
	98.8	1.0		
	101.3			
	99.7			
Impurities C	100.3	0.8	0.9	100.3
	101.7			
	99.7			
	98.5	1.2		
	101.5			
	99.8			
	101.5	0.7		
	99.7			
	100.2			
Impurities D+E	99.8	0.6	0.5	100.1
	101.2			
	100.3			
	99.6	0.8		
	101.4			
	99.7			
	99.9	0.2		
	99.3			
	99.5			
Impurities F	99.3	0.9	0.6	100.2
	99.9			
	101.4			
	101.1	0.5		
	100.2			
	100.0			
	100.7	0.4		
	99.9			
	99.6			

The overall mean recovery was 99.9%. The recovery results of related substances also presented in Table 3, indicating that the method is accurate for the determination of the related substances.

Calibration Curves

The data were subjected to the statistical analysis using a linear-regression model, the standard deviation of slope and intercept are calculated. The linear regression data for the oxycone and its related substances is presented in Table 4. The method was found to be linear with correlation coefficients (R^2) of 0.998-0.999 for oxycodone and its realated substances.

Table 4: Linear range, coefficient of correlation, slope and intercepts for oxycodone and its related substances

Component	Linear range	R^2	Formula
Oxycodone	2-20	0.998	y=25000x+9266
Impurity A	0.69-11.02	0.999	y=26073x+21.71
Impurity B	0.69-11.08	0.999	y=15459x-131.9
Impurity C	0.68-10.88	0.998	y=26773x-4141
Impurity D+E	4.08-20.4	0.999	y=20290x-8410
Impurity F	1.50-24.08	0.999	y=23754x-5512

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Solutions of oxycodone and its related substances containing each at different concentration were prepared and chromatographed. The LOD concentration was that concentration yielding a signal-to-noise ratio of at least 3. The LOQ concentration was that concentration yielding a signal-to-noise ratio of at least 10. Table 5 provides the determined LOD and LOQ values for oxycodone and its related substances.

Table 5: Relative retention time and LOD and LOQ concentrations for components

Components	LOQ (µg/ml)	LOD (µg/ml)	Retention time (min)	Relative retention time (RRT)
Impurity A	0.25	0.06	10.78	0.38
Impurity B	0.30	0.09	17.57	0.66
Oxycodone	0.30	0.08	25.97	1
Impurity C	0.2	0.06	28.71	1.14
impurity（D+E）	0.13	0.04	29.86	1.18
Impurity F	0.20	0.06	54.46	2.4

3.2.4 Stability

The analytical solutions and standard solutions have been shown to be stable for at least 1 week when stored at room temperature. Additionally, the standard solutions have been shown to be stable while in use for assays for at least 24 h. During the period of experiment, no degradation products were observed.

Conclusion

The gradient HPLC method was used in the separation of oxycodone from its related substances. The developed method was found to be accurate, precise, sensitive, linear and stability-indicating for the determination of oxycodone and its related substances in the oxycodone and acetaminophen capsule over the entire stage of investigating process. The method is therefore suitable for the determination of oxycodone and its related substances in oxycodone and acetaminophen capsule as well as pharmaceutical formulations.

REFERENCES

Davis M and Tailcock A (2001). Modified-release opioids [J]. *European Journal of Palliative Care* **8** 142–146.

Davis M and Wilcock A (2001). Modified-release opioids [J]. *European Journal of Palliative Care* **8** 142–146.

Davis MP, Varga J and Dickerson D *et al.*, (2003). Normal-release and controlled-release oxycodone: pharmacokinetics, pharmacodynamics, and controversy [J]. *Support Care Cancer* **11** 84–92.
Leow KP, Cramond T and Smith MT (1995). Pharmacokinetics and pharmacodynamics of oxycodone when given intravenously and rectally to adult patients with cancer pain [J]. *Anesthesia & Analgesia* **80** 296–302.
Maddocks I, Somogyi A and Abbott F *et al.*, (1996). Attenuation of morphine-induced delirium in palliative care by substitution with infusion of oxycodone [J]. *Journal of Pain and Symptom Management* **12** 182–189.
Poyhia R, Vainio A and Kalso E (1993). A review of oxycodone's clinical pharmacokinetics and pharmacodynamics [J]. *Journal of Pain and Symptom Management* **8** 63– 67.
Ripamonti C and Bruera E (1997). CNS adverse effects of opioids in cancer patients [M]. Guidelines for treatment. *CNS Drugs* **8** 21–37.