North-West University (Potchefstroom Campus), Faculty of Health Sciences, Unit for Drug Research and Development, Potchefstroom, South-Africa

UV Spectrophotometric method for the identification and solubility determination of nevirapine

N. Stieger, W. Liebenberg, J.C. Wessels

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Nicole Stieger, Faculty of Health Sciences, Unit for Drug Research and Development, North-West University, University Street, Building G02, 2520 Potchefstroom, South Africa
nicole.stieger@nwu.ac.za

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An UV spectrophotometric method was developed for the quantitative determination of nevirapine in water, methanol and 0.1 N HCl. It was found that qualitative determination is also possible, due to a peak-shift observed with 0.1 N HCl. The solubility at 37 °C, for both the anhydrous and Hemi-hydrated forms of nevirapine, was determined in each of the three solvents.

Nevirapine is an anti-retroviral drug used in the treatment of HIV infection. It is a non-nucleoside reverse transcriptase inhibitor of the dipyridodiazepinone class. To minimize viral resistance, this drug is almost exclusively used in combination therapy (Beers et al. 1999; O'Neil et al. 2006).
Although complete solubility and permeability data for nevirapine are lacking, the FDA classifies the drug as a class II (high permeability, low solubility) drug (Lindenberg et al. 2004). Moffat et al. (2004) and O'Neil et al. (2006) specify water solubility at neutral pH to be ~1 mg/ml, whilst stating that the drug is highly soluble at pH < 3.
The USP (accessed online 2009) describes an HPLC method for quantification, but it has been reported that UV spectroscopy is also a fast, reliable and accurate technique for the determination of nevirapine (Anbazhagan et al. 2005; Sarkar et al. 2006). The solubility and UV absorption of two known forms of nevirapine – anhydrous and hemi-hydrate – were investigated in this study.
The spectrum of nevirapine dissolved in either water or methanol, shows a peak of maximum absorbance at 283 nm. If 0.1 N HCl is used as solvent, the nevirapine peak of maximum absorbance shifts to 313 nm (Fig.). This peak-shift associated with solvents can be a useful method for nevirapine identification, especially if more sophisticated equipment is not available. Currently, the USP (2009) only describes two methods of identification for nevirapine, namely IR absorption and HPLC assay (peak retention times when compared to a standard).
The solubility at 37 °C of each form in the three solvents, is given in the Table. Although both forms are approximately 40 times more soluble in 0.1 N HCl than in water, the anhydrous form is about 1.7 times more soluble than the hemi-hydrate in each of these aqueous media. Methanol is the exception, in that both forms are equally soluble. (Table)

Experimental

A Shimadzu UV-1800 (Japan) UV spectrophotometer was used for all experimental work pertaining to the results reported in this article.
Nevirapine (anhydrous) was purchased from Cipla (Mumbai, India, batch number: 1001003). The hemihydrate was prepared by recrystallization from ethanol containing 70% water. The physico-chemical properties of both forms were confirmed by DSC, DTG, KF, IR and XRPD analyses. Particle sizes were determined with a Galai CIS-1 (Israel) system. The mean particle size by volume, for both forms, was smaller than 25 μm.
All chemicals used were analytical grade and water was prepared with a Millipore™ MilliQ® Ultrapure Water Purification System (USA).
Wavelength of maximum absorbance: Dilute solutions of nevirapine were scanned from 200–800 nm to determine the wavelength/s of maximum absorbance.
Standard curve: Nine concentrations of nevirapine (6.0, 5.0, 4.0, 2.0, 1.0, 0.5, 0.25, 0.125 and 0.0625 mg/100 ml), in the three respective solvents, were prepared in order to construct a standard curve.

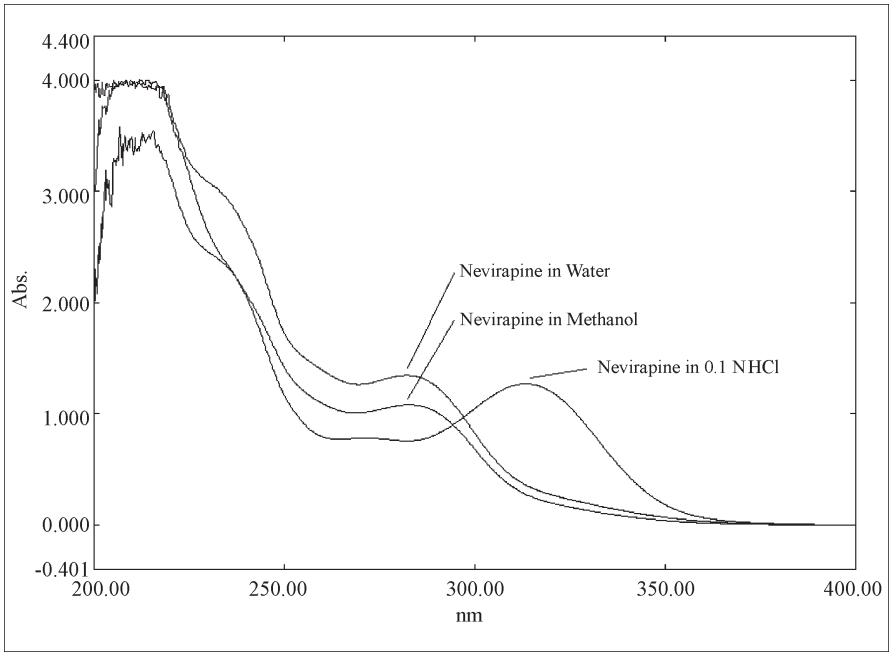

Fig.: UV spectra of nevirapine in water, methanol and 0.1 N HCl

Table: Solubility data for nevirapine in water, 0.1 N HCl and methanol

	R^2- calibration curve	Equation for calibration curve	Solubility at 37 °C (mg/100 ml)	Relative solubility of form in solvent	Relative standard deviation (%)
Anhydrous in water	0.9938	$y = 0.2871x + 0.0264$	9.76	1.68	1.7
Hemi-hydrate in water			5.82	1.00	1.4
Anhydrous in 0.1 N HCl	0.9999	$y = 0.3109x + 0.0042$	394.38	1.69	0.96
Hemi-hydrate in 0.1 N HCl			233.38	1.00	1.6
Anhydrous in methanol	0.9996	$y = 0.2803x - 0.0035$	938.81	1.00	0.52
Hemi-hydrate in methanol			939.40	1.00	0.59

Solubility: Six 20 ml amber test tubes for each nevirapine form and solvent were filled with solvent and an excess of nevirapine powder. The test tubes were fixed to a rotating axis (54 rpm) submerged in a water bath at 37 ± 2 °C. After 24 h, the solutions were filtered through 0.8/0.2 μm Supor® membranes to exclude all remaining solids. 24 h were deemed to be a sufficient period for equilibrium of solution to be reached. After dilution the concentration of the filtrates was determined.

- Dilution for nevirapine in water: 5 ml to 10 ml.
- Dilution for nevirapine in 0.1 N HCl: 1 ml to 100 ml.
- Dilution for nevirapine in methanol: 1 ml to 10 ml, then 1 ml to 25 ml (resulting in a 1 ml to 250 ml dilution).

The methanol solution was diluted in two steps to reduce the amount of solvent used.

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