CAPSULES notes and applications from BASi

September 2003

374

Sildenafil

A LC/MS/MS Determination of Sildenafil and its Primary Pharmacologically Active Metabolite, Desmethyl Sildenafil, in Human Plasma

Method Profile

Liquid-liquid extraction for fewer interferences
Postcolumn switching to reduce MS contamination and increase method ruggedness
Less than 0.5 mL plasma required
Rapid sample turnaround
Wide linear range
LC/MS/MS maximizes specificity

Sensitivity

Sildenafil LOQ: 1.0 ng/mL
Desmethyl Sidenafil LOQ: 1.0 ng/mL

BASi has incorporated a LC/MS/MS method for the simultaneous quantitation of sildenafil and its major metabolite, desmethyl sildenafil, in human plasma. The method has been rigorously validated under GLP over a range of 1-500 ng/mL for sildenafil and 1-300 ng/mL for desmethyl sildenafil. The method requires 0.2 mL of plasma and is highly selective for the analytes.

Sildenafil is used in the treatment of erectile dysfunction, acting as a potent and selective inhibitor of cGMP-specific PDE5, the predominant isozyme that metabolizes cGMP in the corpus cavernosum of the penis. Many likely users of sildenafil suffer from a variety of physiological conditions, and it is expected that patients receiving sildenafil may be candidates for treatment with other drugs. Sildenafil is primarily metabolized by the cytochrome P450 3A4 (major route) and 2C9 (minor route) hepatic microsomal isoenzymes, which convert it to an active N-desmethyl metabolite that has been shown to possess 50% of the parent drug's potency for inhibiting PDE5. Inhibitors of the P450 3A4 pathway may increase the plasma concentrations of sildenafil and therefore its pharmacological effect, possibly increasing the incidence of adverse events. This assay is designed to allow determination of sildenafil and desmethyl sildenafil concentrations in human plasma alone or when co-administered with other drugs.

To increase method ruggedness, sample preparation makes use of liquid-liquid extraction to provide cleaner samples. A postcolumn switching valve is used so that unwanted plasma components are sent to waste and only the analytes of interest are sent to the MS, thus increasing throughput without compromise of the MS signal over time. The method's 6.5-minute cycle time allows analysis of 200 samples in an overnight sequence. The result? Higher quality data with shorter turnaround times and fewer repeated samples due to rejected chromatograms and failing runs.

The sildenafil method is fully validated for the analysis of sildenafil and desmethyl sildenafil in heparinized human plasma. A partial validation for other anticoagulants can be performed at no charge. Validation for the simultaneous analysis of co-administered drugs is available upon request.

Method Performance Data

A nine-point standard curve (SC) was run during the validation. The data show that linearity, precision, and recovery are within the desired limits, as can be seen with the sampling of data contained in ***T1*** and ***T2***.

Quality control (QC) samples are generated by spiking plasma with the analytes at levels simulating those expected in real samples. Four QC levels (one low-level, two mid-range, and one high-level) were injected (n = 6) in each of three runs. Accuracy and precision for these samples in three consecutive runs are shown in ***T3***. Recovery was greater than 89% for all samples tested.

Additional testing subjected selected samples to normal sample handling challenges, including stability in plasma, stability of extracted samples, the effect of freeze-thaw, and dilution of over-curve samples. In all cases, the method maintained the requisite precision and accuracy for sildenafil and its metabolite.

T1. Between-run (n = 3) accuracy and precision for injections of extracted standards.

	Sildenafil		Desmethyl sildenafil	
Sample name	**Mean**	**CV**	**Mean**	**CV**
SC-1 ng/mL	101%	2.0%	102%	1.5%
SC-3 ng/mL	100%	5.8%	95%	4.6%
SC-7.5 ng/mL	98%	1.4%	99%	1.2%
SC-10 ng/mL	94%	5.0%	98%	2.7%
SC-30 ng/mL	104%	5.5%	97%	6.4%
SC-75 ng/mL	100%	1.8%	102%	5.0%
SC-100 ng/mL	100%	5.0%	104%	7.6%
SC-300 ng/mL	107%	5.4%	103%	4.2%
SC-500 ng/mL	99%	9.4%	nd*	nd*

T2. Typical linearity data for standard curve (1-500 ng/mL and 1-300 ng/mL) of sildenafil and desmethyl sildenafil.

Sample name	r^2
Sildenafil	0.9909
Desmethyl sildenafil	0.9967

T3. Between-run (n = 18) accuracy and precision for injections of extracted spiked samples (QC samples).

	Sildenafil		Desmethyl sildenafil	
Sample name	**Mean**	**CV**	**Mean**	**CV**
QC-1 ng/mL	99%	11.2%	98%	9.1%
QC-3 ng/mL	93%	5.9%	93%	4.8%
QC-30 ng/mL	98%	4.4%	89%	7.9%
QC-300 ng/mL	nd*	nd*	102%	6.2%
QC-500 ng/mL	101%	3.4%	nd**	nd**

*not determined (QCs only at low, mid-, and highest levels)
**not determined (desmethyl sildenafil standard curve range is 1-300 ng/mL)

Mass chromatograms of sildenafil and desmethyl sildenafil. Chromatographic resolution is obtained in less than four min. Each analyte is determined by LC/MS/MS. In the first stage of the quadrupole, the mass spectrometer isolates the molecular ions as shown in the mass chromatograms. Then the added specificity of MS/MS detection is used to identify and quantify the analytes by their unique product ions. Thus specificity and low limits of detection are obtained with a high degree of accuracy and precision. An internal standard is used to add method ruggedness by correcting for normal variations that happen during sample processing.

The chromatograms show a significant signal is obtained at the lower end of the standard curve (1.0 ng/mL), through the upper end of the curve (500 ng/mL for sildenafil, 300 ng/mL for desmethyl sildenafil). The blank baseline shows the method is interference-free, improving precision and accuracy of the method as well as user confidence in the high quality of the results.