Determination of the Vasodilator Isosorbide-5-Mononitrate in Human Plasma using GC/MS with Electron Capture Negative Ion Chemical Ionization

Application Note

Drug Testing

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Introduction

The 1998 Nobel Prize in Physiology and Medicine was awarded for work demonstrating "Signal transmission by a gas that is produced by one cell, penetrates through membranes and regulates the function of another cell…." This work, which elucidated the role of nitric oxide (NO) in the dilation of blood vessels, has lead to the development of a series of vasodilatory drugs for use as anti-anginal and anti-impotence medications (e.g., Viagra®). It is perhaps ironic that the best known of the anti-anginal medications, nitroglycerin, apparently had been prescribed to no lesser a patient than the founder of the Prize, Alfred Nobel.

An example of a more recent vasodilator typical of the organic nitrate agents is the anti-anginal agent 1,4:3,6-dianhydro-D-glucitol 5-nitrate, or isosorbide-5-mononitrate (Figure 1). Isosorbide-5-mononitrate and the other organic nitrate anti-angina medications require different dosing approaches than most other chronically used drugs. Usually, drug dosage is designed to maintain plasma concentrations in excess of that ensuring minimum efficacy. However this approach appears inappropriate for organic nitrate drugs, because the patient develops nitrate tolerance.

One of the difficulties in designing an optimal dosing interval is sensitive detection of the organic nitrate. This Application Note demonstrates a successful approach to sensitive detection and accurate quantitation of one member of this class of drugs, the isosorbide-5-mononitrate (IS5MN), using electron capture negative ion chemical ionization (ECNI). It also demonstrates an important advantage of ECNI: greater selectivity. IS5MN contains electrophilic oxygen atoms which provide a high cross section for electron capture relative to the biogenic interferences present in the plasma. This selectivity, combined with high sensitivity, allows detection and quantitation of electrophilic compounds in complex matrices such as biological samples.

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Experimental

A 1-ml aliquot of the plasma sample is spiked with *p*-nitrobenzyl alcohol, which serves as an internal standard (IS), at 250 ng/ml. One milliliter of deionized water is added and the sample thoroughly mixed before liquid–liquid extraction with ethyl acetate (solvent:aqueous, 10:2 v/v). The sample is then centrifuged, the upper layer removed and transferred and then evaporated to dryness. The residue is immediately derivatized by adding BSTFA+1% TMCS in dry pyridine (1:1 v/v). The trimethyl-silyl derivatives of the IS5MN and IS are shown in Figure 1. The excess agent is evaporated and the sample reconstituted in 200 µl of dry hexane.

GC-MS-ECNI analysis of the sample is made according to the instrumental parameters given in Table 1.

Table 1. GC and MSD Instrumental Parameters

GC Injection Parameters	
Autosampler	7683 ALS
Injection	1-µl
Mode	Pulsed Splitless
Injector temperature	250°C
GC Oven Parameters	
GC column	J&W DB1701 15m, 0.25 mm i.d., 0.25 µm film
Carrier gas	Helium
Column flow	1.2 ml/min, constant flow
Initial temperature	60°C
Ramp rate	30°C/min
Final temperature	260°C
Transfer line	280°C
MSD Parameters	
Mode	NCI
Buffer gas	Ammonia
Mass Flow Controller setting	20%
Source temperature	150°C
Quadrupole temperature	103°C
Tune parameters	NCI autotune

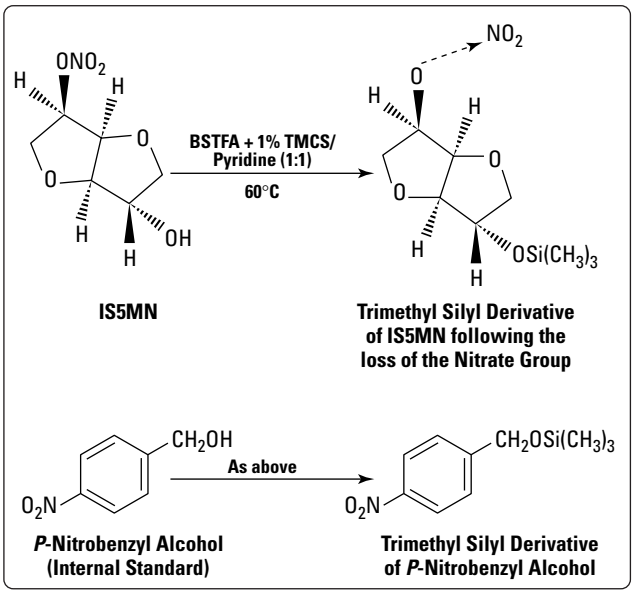

Figure 1. Chemical structures of the Isosorbide-5-mononitrate (IS5MN) and *p*-nitrobenzyl alcohol (IS) and the derivatization scheme.

Results

The mass spectra of the trimethyl-silyl derivatives of the IS5MN and IS show very little fragmentation (Figures 2 and 3) as expected in electron capture negative chemical ionization (ECNI). Quantitative analysis used selected ion monitoring ions of 217 *m/z* and 225 *m/z*, respectively. A calibration curve, constructed using standards of nominally 10, 50, 100, 200, 500, 750, 1000 ng/ml concentrations, was linear over the entire range with an r^2 value of 0.996 (Figure 4).

To test the accuracy and precision of the method, intraday and interday batches were analyzed. These results, shown in Tables 2 and 3, demonstrate that the accuracy achieved was better than ± 6% of the true value and the reproducibility better than 6.4% for IS5MN concentrations from 20 to 800 ng/ml. The quantitation limit for IS5MN is nominally 10 ng/ml (Figure 5). This method has proved extremely useful in evaluating the pharmacokinetics and bioequivalence of a number of IS5MN preparations in human subjects.

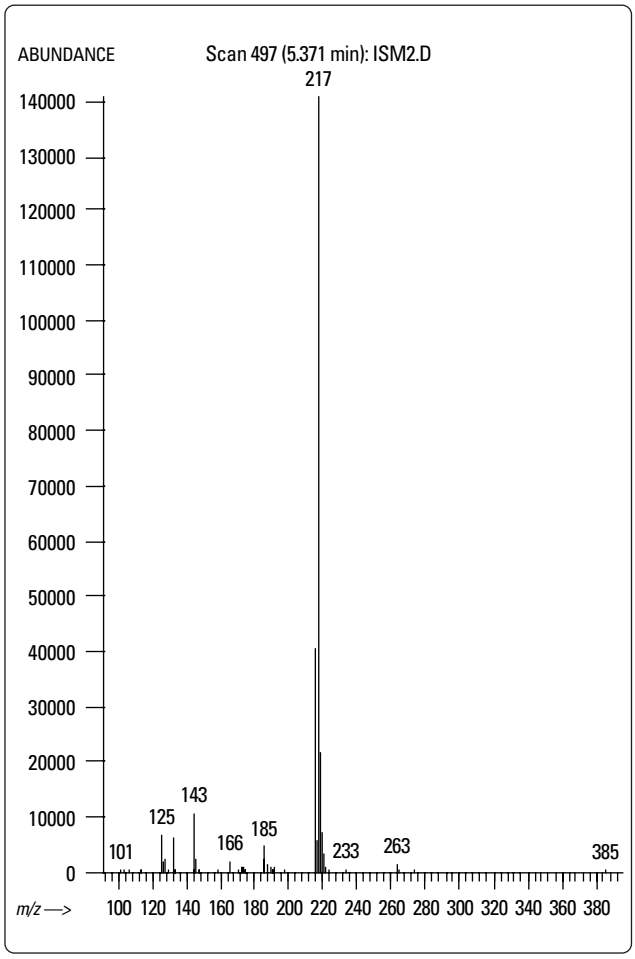

Figure 2. Electron capture negative ion chemical ionization mass spectrum of the trimethyl-silyl derivative of isosorbide-5-mononitrate from 100 to 400 amu using ammonia buffer gas.

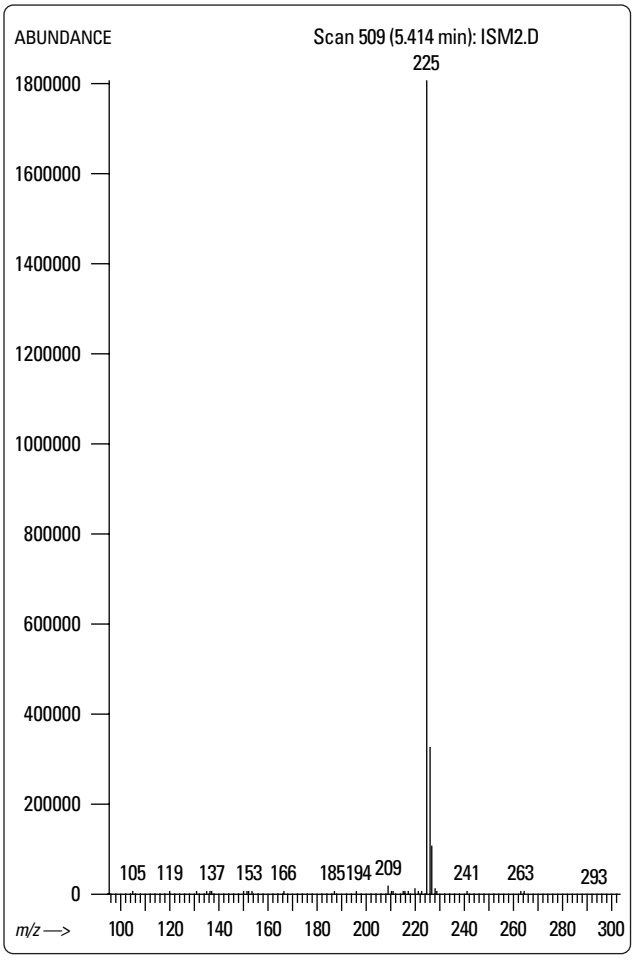

Figure 3. Electron capture negative ion chemical ionization mass spectrum of the trimethyl-silyl derivative of *p*-nitrobenzyl alcohol internal standard from 100 to 400 amu using ammonia buffer gas.

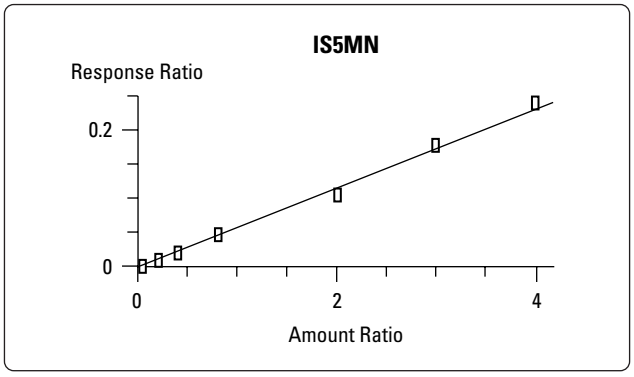

Figure 4. Calibration curve for IS5MN from 10 ng/ml to 1000 ng/ml, $r^2 = 0.996$.

Table 2. Summary of intra-batch quality control data (n=6)

Control Concentration (ng ml^{-1})	Mean Determined Value (ng ml^{-1})	Imprecision		Accuracy (Mean as % of Actual Concentration)
		Standard Deviation	Coefficient of Variation (%)	
20.00	20.90	1.33	6.38	104.50
150.09	142.14	0.95	0.67	94.70
399.24	396.28	15.06	3.80	99.26
795.32	807.77	31.49	3.90	101.57

Table 3. Summary of inter-batch quality control data (n=6)

Control Concentration (ng ml^{-1})	Mean Determined Value (ng ml^{-1})	Imprecision		Accuracy (Mean as % of Actual Concentration)
		Standard Deviation	Coefficient of Variation (%)	
20.00	20.94	0.95	4.54	104.70
150.09	146.86	8.27	5.63	97.85
399.24	421.16	16.25	3.86	105.49
795.32	824.00	28.37	3.44	103.61

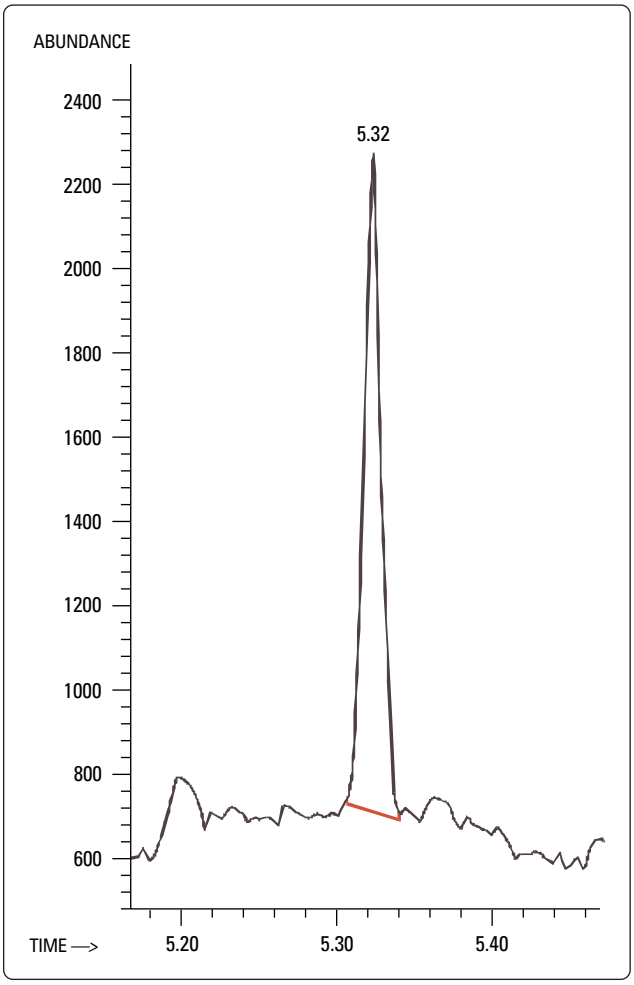

Figure 5. Extracted ion chromatogram for ECNI-SIM acquisition of a 10 ng/ml IS5MN plasma standard. Displayed is the 217 amu ion and ± 0.15 min of the expected retention time for the analyte. The analyte shows an RMS signal-to-noise greater than 100 even at a concentration near the quantitation limit.

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