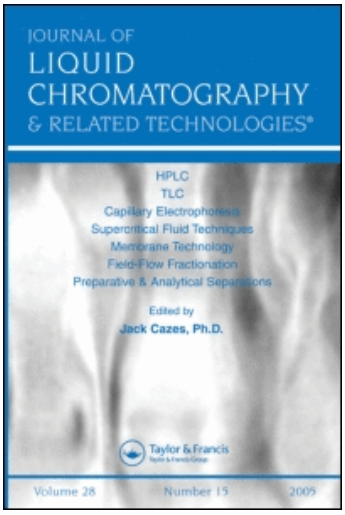

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A SENSITIVE AND SPECIFIC METHOD FOR ASSAY OF SERTINDOLE AND ITS METABOLITES IN HUMAN, RAT, DOG, AND MOUSE PLASMA USING HPLC WITH TANDEM MASS SPECTROMETRIC DETECTION

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ABSTRACT

A sensitive and specific assay was developed for the concurrent determination of sertindole, a new antipsychotic agent, and its two major metabolites, Lu 28-092 and Lu 25-073, in plasma. The method employs reverse-phase high performance liquid chromatography (HPLC) with tandem mass spectrometric detection. Chromatographic separation is necessary because sertindole and the Lu 28-092 metabolite differ by only two mass units. Results comparable to those found in human plasma were obtained in dog, rat, and mouse plasma, indicating the method is robust and relatively insensitive to matrix constituents.

INTRODUCTION

Sertindole is a new antipsychotic drug with demonstrated efficacy in patients with schizophrenia, but without the adverse effects associated with conventional therapeutic agents. Efficacy is thought to be derived from the drug's selective effects on mesolimbic but not nigrostriatal dopaminergic neurons.[1-5] Two major metabolites of sertindole are formed *in vivo* after oral administration of the drug: dehydro-sertindole (Lu 28-092) and nor-sertindole (Lu 25-073). Because of the importance of the pharmacokinetic characteristics of these metabolites, they were included in the development of an assay to be used in toxicological studies and clinical trials.

Due to its high potency, sertindole is administered at relatively low doses, resulting in low circulating levels of the parent compound and its metabolites. Therefore, a method with high sensitivity and selectivity is required for the quantitation of these analytes. Initially, a reversed-phase HPLC method with ultraviolet detection was developed. The detection limit was approximately 1 ng/mL, for 1 mL of plasma extracted. Multiple liquid/liquid extraction steps were required for sample clean-up and the method suffered from poor reproducibility and tedious work-up procedures. Later, a major improvement in detection limits was achieved using normal-phase HPLC separation with fluorometric detection.

This method was further improved by Tzeng et al.,[6] using a simplified solid-phase extraction step; the fluorometric method had a lower limit of quantitation of 25 pg/mL, with a linear dynamic range up to 4 ng/mL. However, the method was susceptible to interferences from the serum or plasma matrix. Moreover, the Lu 28-092 metabolite eluted as a broad peak in this system, and the Lu 25-073 metabolite was a nonquantifiable late eluting peak.

The method described in this paper was developed in order to measure all three analytes in a single chromatographic run. The method was validated in human plasma and that of three animal species, the rat, dog, and mouse. The assay is based on reverse-phase HPLC with tandem mass spectrometric (MS/MS) detection. The sample clean-up procedure is similar to that described by Tzeng et al.,[6] but includes modifications to permit the co-extraction of the two metabolites as well as the parent compound.

Our experiments demonstrate that atmospheric pressure ionization with tandem mass spectrometry as a detection technique results in good sensitivity for the analytes, along with a short analytical run time which provides high sample throughput capability.

MATERIALS AND METHODS

Reagents and Chemicals

Absolute ethanol was obtained from McCormick (Weston, MO). Glacial acetic acid 99.99% was obtained from Aldrich (Milwaukee, WI). All other chemicals and solvents were HPLC grade obtained from EM Science (Gibbstown, NJ). Eluent and reconstitution solvents were filtered through a 0.2 μm-pore nylon membrane before use. Reference materials: Sertindole was synthesized by Abbott Laboratories; Lu 28-092, Lu 25-073, and Lu 26-009 (internal standard) were obtained from Lundbeck A/S (Copenhagen).

Extraction

Analytes were extracted from plasma using solid-phase extraction. The C_8 solid-phase extraction column (Bond Elut 200 mg, Varian Associates, Harbor City, CA or Isolute IST-EC, Jones Chromatography, Lakewood, CO) was preconditioned with two washes of acetonitrile (2 mL each), then three washings of methanol (3 mL each), followed by two washes of purified water (2 mL each) (MilliQ UV system, Millipore Corp., Bedford, MA). Care was taken to avoid drying the bed, which can disrupt analyte/ solid-phase binding interactions. Plasma was pipetted into a clean borosilicate test tube; extraction volumes were 1000, 500, 200, and 100 μL for human, dog, rat, and mouse plasma, respectively. Samples were supplemented with a phosphate buffer (10 mM dibasic potassium phosphate; pH 8.5) to aid in sample transfer. Then 50 μL of a 100 ng/mL solution of the internal standard (Lu 26-009) in ethanol was added. The tube was vortexed briefly to blend the internal standard and matrix.

The sample was then loaded onto the preconditioned solid-phase column using a gentle vacuum (1 to 2 min residence of matrix on the column). The extraction column was washed with three rinses of purified water (2 mL each) followed by three rinses of acetonitrile (1 mL each). The column was then dried by continuous application of a vacuum, drawing air through the column for 1 to 2 min. Analytes trapped onto the column were eluted with two 1-mL aliquots of acidified methanol (2% glacial acetic acid, 98% methanol; v/v). The eluate was collected into a silanized borosilicate tube and evaporated to dryness under a gentle stream of air or nitrogen, in a water bath maintained at 25° to 35°C. The dried residues were reconstituted into 50 μL of the mobile phase, transferred to a 300-μL silanized micro vial, capped, and centrifuged at approximately 3000 rpm for about 5 min. The reconstituted extract was maintained at 5°C or less until injection.

Chromatography

Analytes were separated by reverse-phase chromatography on a YMC basic column (150 x 2.1 mm; 5 micron, YMC Inc., Wilmington, NC). The analytic column was protected by a guard cartridge. The mobile phase was a mixture (v/v) of 42% acetonitrile and 58% of a 100 mM solution of ammonium acetate in purified water (MilliQ UV). The pH of the mobile phase was adjusted to 6.8 with glacial acetic acid. The mobile phase was pumped using a syringe pump (Model 500D, Isco, Lincoln, NE) at a flow rate of approximately 400 μL/min. Column back pressure ranged between 1500 and 2000 psi at this flow rate. Samples (20 μL) were injected using a CMA-200 refrigerated microsampler (CMA/Microdialysis AB, Stockholm, Sweden). Samples were maintained at a temperature of approximately 5 5°C until injection. Run time was approximately 6 min.

Mass Spectrometric Detection

Analytes in the HPLC effluent were detected and measured using the API III mass spectrometer (SCIEX, Thornhill, Ontario, Canada). The chromatographic system was coupled to the mass spectrometer using the atmospheric pressure ionization source (Heated Nebulizer, SCIEX). Analytes were ionized in the positive ion mode at a source temperature of approximately 450°C. Nitrogen was used both as the nebulizing gas (approximate pressure 80 psi) and as the auxiliary sheath gas (flow rate 2 to 3 L/min). Nitrogen for nebulizing and auxiliary flow was generated by a gas generator (Nitrox NG-4000, Peak Scientific, Buffalo Grove, IL). The purity of the nitrogen was 95% to 99%, depending on the flow rate. The curtain gas was ultra high purity nitrogen (99.999%, AGA Gas Inc., Maumee, OH), at a flow rate of approximately 1.8 L/min. Analyte specific detection was performed in the multiple reaction monitoring mode. Ultra high purity argon (99.999%, AGA Gas Inc.) was used as the reagent gas for collision-activated decomposition of selected parent ions. Data was acquired using a dwell time of 200 ms per channel, which permitted about 20 to 30 scans across chromatographic peaks which generally had peak widths of 40 to 60 seconds. Before each assay batch was loaded in the autosampler for analysis the instrument was checked for proper performance by injecting a reference standard containing all the analytes of interest (approximately 2 ng each injected on the column).

Preparation of Standard and Quality Control Samples

Reference solutions of sertindole and Lu 28-092 were prepared as ethanolic solutions at approximately 1.0 mg/mL. The reference solution of Lu 25-073 (supplied as the fumarate salt) was prepared in methanol because of the poor

solubility of the salt in ethanol. Appropriate volumes of each solution were combined and diluted with methanol to yield a spiking solution containing approximately 10 μg/mL each of sertindole, Lu 28-092, and Lu 25-073. Spiking solutions were prepared independently for standards and quality control (QC) samples.

For the standards, a stock solution of analytes in human plasma (approximately 200 ng/mL) was prepared by spiking the matrix with the reference solution. This stock solution in plasma was then serially diluted to obtain about 10 standard levels, ranging in concentration from 0.1 to 50 ng/mL. QC samples were prepared similarly from a separate weighing, at three concentration levels: approximately 0.7, 10, and 26 ng/mL in human plasma; they were designated QC low, QC mid, and QC high, respectively. The concentrations of standards and quality control samples in the animal matrices were different, reflecting differing extraction volumes and other analytical considerations. (Actual concentrations of the QCs in each matrix appear in Table 2 footnotes.) The volume of plasma extracted was different for the animal species because generally less plasma is available for analysis.

Quantitation Method

Peak areas of sertindole, Lu 28-092, Lu 25-073, and the internal standard were determined using McQuan software (version 1.2, SCIEX). Calibration curves for each analyte were derived from the ratio of analyte peak area to internal standard area and using least-squares linear regression to obtain the relationship of this ratio to the theoretical concentration at each standard level. A weighting of 1/x (where x was the concentration of a given standard level) was generally found to give an optimal fit to the concentration/response data; in some case a weighting of $1/x^2$ gave a better fit. The residuals were examined for outliers, which were excluded, and the curve was recomputed to obtain a better fit. Concentrations of the QC samples were calculated from the regression curve using the observed response ratio.

RESULTS AND DISCUSSION

Choice of Analyte-Specific Reactions

A single-stage scan of the ions produced from an infusion of sertindole, Lu 28-092, Lu 25-073, and Lu 26-009 showed only the protonated molecular ions and the characteristic chlorine isotope pattern for these substances. The product ion spectra

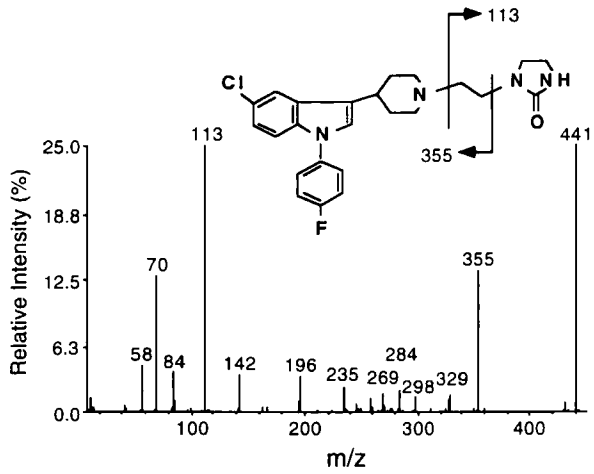

Figure 1. Fragmentation of the $[M + H]^+$ ion (m/z 441) of sertindole.

(MS/MS) of these protonated molecular ions were obtained using argon reagent gas for collision-activated decomposition. Figure 1 shows the product ion spectrum of sertindole. The major fragmentation pathway is the retention of the imidazolidinone moiety (m/z 113 for sertindole). Fragmentations for Lu 28-092 and the internal standard Lu 26-009 were similar (spectra not shown), with appropriate shifts in parent and product ion masses. The fragmentation for Lu 25-073 is different because the molecule does not contain the imidazolidinone moiety. The major fragment ion is at m/z 84. This fragment is thought to arise from opening of the piperazine ring. The following channels were chosen in the multiple reaction monitoring mode (MRM) for peak detection and quantitation: m/z 441→113 for sertindole, m/z 439 →111 for Lu 28-092, m/z 329→84 for Lu 25-073, and m/z 437 →113 for the internal standard. The resolution of the mass filters was reduced for the MRM mode in order to improve ion transmission efficiency.

Specificity

Chromatograms obtained from the injection of a reference solution containing approximately 100 ng/mL of each analyte revealed small peaks in the Lu 28-092 channel. The peaks correspond to the cross-channel talk from the internal standard and sertindole peaks. Sertindole, Lu 28-092, and the internal standard differ by only two mass units; therefore, under the lower resolution conditions employed in the MRM mode there is a small degree of cross talk. Specificity accomplished purely by

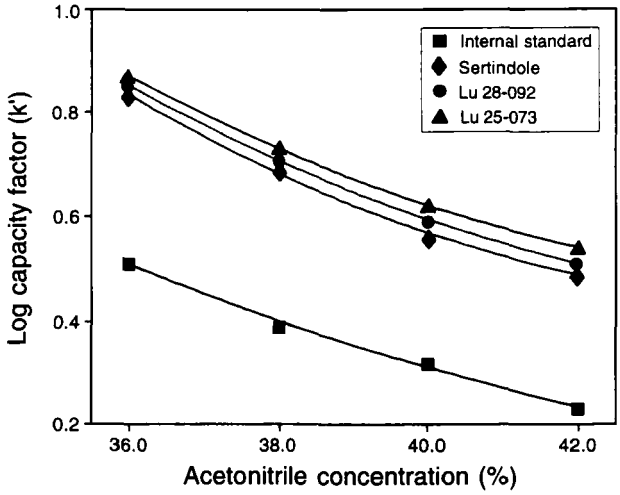

Figure 2. Effect of organic strength on resolution of Lu 26-009 (internal standard), sertindole, Lu 28-092, and Lu-073 at pH 6.0.

mass selection (increasing resolution) would be achieved at the cost of sensitivity. Therefore, these compounds needed to be separated chromatographically. To achieve rapid chromatographic separation of the analytes, we examined the effect of solvent strength and pH on resolution of the analytes on the YMCbasic chromatographic column.

Figure 2 shows the change in the log capacity factor (k') as a function of organic solvent content in the mobile phase at a constant pH of 6.0. The plot demonstrates that increasing the organic solvent strength to about 42% decreased the run time without affecting resolution. A solvent strength of 42% acetonitrile and 58% aqueous 100 mM ammonium acetate was chosen as the optimal concentration to achieve short run times without the peaks eluting too close to each other.

Figure 3 shows a plot of the retention time of the four analytes as a function of mobile phase pH. The organic strength of the mobile phase was held constant at 42% acetonitrile. The pH of the mobile phase had a significant effect on the resolution and elution order of the analytes as demonstrated in the plot. A pH of 6.8 was selected, as it enabled optimal resolution of all the analytes while maintaining a short chromatographic run time (less than 6 min).

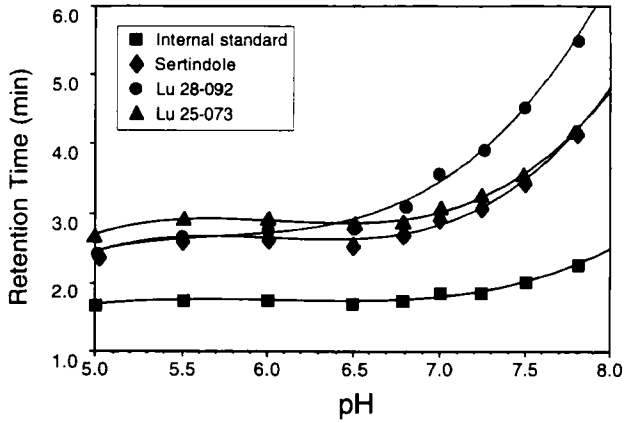

Figure 3. Effect of pH of mobile phase on resolution of Lu 26-009 (internal standard), sertindole, Lu 28-092, and Lu 25-073 at constant organic strength. Composition of the mobile phase was 42% acetonitrile and 58% 100 mM aqueous ammonium acetate in water (v/v).

Ion Current Stability

Multiple injections of a standard reference solution containing 100 ng/mL of each analyte were made to investigate signal stability and the reproducibility of the atmospheric ionization process. The relative standard deviation (RSD) for the ratio of analyte to internal standard (determined from 25 injections) was 7.5% for sertindole, 7.9% for Lu 28-092, and 10.2% for Lu 25-073. No significant trend or drift in the ratio of analyte to internal standard was observed; however, the signal intensity for all analytes decreased after multiple injections. The reason for this drop in signal intensity over time is not apparent.

Limit of Quantitation

The limit of quantitation (LOQ) was determined from standard curves obtained in a study containing eight assay batches and spanning a period of approximately 6 months. The sertindole peak for standard level-1 (approximately 0.1 ng/mL) was detected in all eight calibration curves. The Lu 28-092 peak was detected in seven of the eight curves. However, the Lu 25-073 peak for standard level-1 was not observed in three out of the eight calibration curves, so a higher standard level (level-2, approximately 0.2 ng/mL) was considered as the LOQ for Lu

25-073. The area of the sertindole peak for standard level-1 had an average signal-to-noise (S/N) ratio of 17, with an RSD of 21%. The Lu 28-092 peak for standard level-1 had an average S/N of 12.6 and an RSD of 21%. The Lu 25-073 peak at standard level-2 (0.2 ng/mL) had an average S/N of 18 and an RSD of 21%. The relatively high RSD of the signal near the LOQ suggests that it was prudent to allow a generous S/N (greater than 10) at LOQ when using mass spectrometry for quantitation.

The precision and accuracy for these levels (0.1 ng/mL for sertindole and Lu 28-092, and 0.2 ng/mL for Lu 25-073), were found to be acceptable, indicating that these concentrations could be supported as the LOQ of the assay (1 mL of plasma extracted). The average recalculated concentration (expressed as percent of the theoretical concentration) was 93.8%, 101.0%, and 89.5%, for sertindole, Lu 28-092, and Lu 25-073, respectively. The respective RSDs were 10.7%, 10.3%, and 16.4%. Figure 4 shows a typical chromatogram for the 0.1 ng/mL standard level. Because the Lu 25-073 peak was not sufficiently distinguished from the background noise in some batches, the higher standard level of 0.2 ng/mL was adopted.

Results in dog, rat, and mouse plasma were comparable, with the LOQ for sertindole and Lu 28-092 ranging from 1 to 2 ng/mL (smaller volumes of plasma extracted as noted above). The LOQ for Lu 25-073 ranged from 1 to 4 ng/mL. The variation in limits of quantitation between the animal matrices after accounting for different extraction volumes might be due to slight differences in the efficiency of extraction from the different matrices.

Linearity

For sertindole and its metabolites, standard curves of the internal standard ratio versus concentration of the analyte were linear over greater than two orders of concentration, ranging from the LOQ to approximately 50 ng/mL in human plasma and from the LOQ to approximately 200 ng/mL in dog, rat, and mouse plasma. Nonlinearity (mean deviation exceeding 20% of the theoretical concentration at either end of the curve), was observed when the range of the calibration curve was increased substantially above this value (i.e. three orders of magnitude or more).

A weighting scheme of $1/x$ or $1/x^2$ (where x is the nominal concentration of the standard level) was necessary to obtain good accuracy at the low end of the calibration curves. The inter-assay accuracy for recalculated standard concentrations in human plasma ranged from 91.3% to 106.6% for sertindole, 90.8% to 116.6% for Lu 28-092, and 89.5% to 105.2% for Lu 25-073.

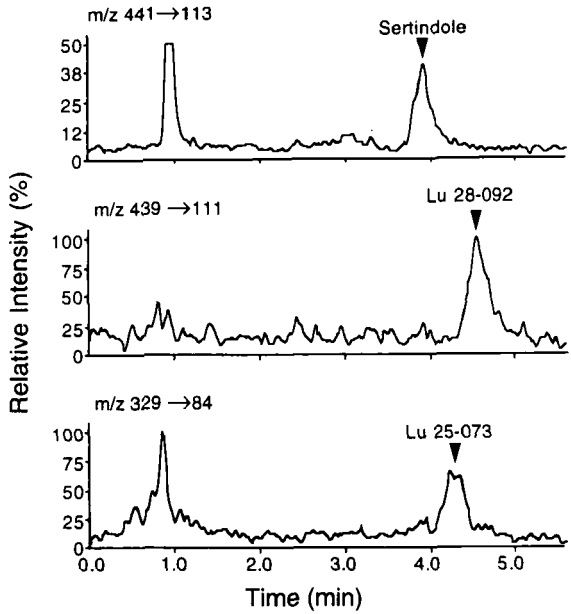

Figure 4. Chromatogram obtained for the 0.1 ng/mL standard in human plasma. This standard level represented the limit of quantitation for sertindole and Lu 28-092. While clearly seen in this chromatogram the peak for Lu 25-073 was not readily identifiable from background in other batches.

Residuals were randomly distributed across the range of the concentration curve, with no significant trend or bias apparent. The RSDs of the recalculated standard concentrations for sertindole, Lu 28-092, and Lu 25-073 averaged 7%, 16%, and 13%, respectively (the mean of RSDs for each standard level).

Precision and Accuracy

Precision and accuracy were determined from the observed concentrations of quality control samples that were assayed along with pharmacokinetic samples from an in house clinical study. Samples were assayed in eight analytical batches spanning a 6-month period. Each batch contained standards, QC samples, and unknowns, with the total number ranging from 80 to 110 samples per assay batch. QC samples at each of the three concentration levels were assayed in triplicate (the first batch had four replicates at each level). Intra-assay statistics were computed from the within-day determination of concentration for the replicate QC samples.

Table 1

Accuracy and Precision of Assays for Sertindole and Metabolites in Human Plasma

	QC Low	QC Mid	QC High
Intra-day accuracy*			
Sertindole	94-122	84-113	88-116
LU 28-092	79-124	71-113	95-145
Lu 25-073	98-127	83-132	92-139
Intra-day precision**			
Sertindole	5.3	5.3	4.5
LU 28-092	15.0	14.0	12.7
Lu 25-073	8.9	9.5	11.2
Inter-day accuracy			
Sertindole	107.7	100.5	104.2
LU 28-092	103.7	95.4	111.4
Lu 25-073	111.9	104.6	111.3
Inter-day precision			
Sertindole	9.2	9.9	8.5
LU 28-092	20.3	19.8	17.2
Lu 25-073	12.6	16.5	16.6

Note: Nominal concentrations of quality control samples were: 0.65 ng/mL for QC low, 10 ng/mL for QC mid, and 26 ng/mL for QC high. There were small differences in the concentration for each compound.
* Range of intra-day means.
** Average of intra-day RSDs.

QC results from all assay batches were pooled to obtain inter-assay (between-day) statistics. Accuracy is reported as the mean concentration of replicate QC determinations, and is expressed as percent of the theoretical concentration. Precision is reported as the RSD of replicate quality control measurements. Table 1 shows the precision and accuracy of the assay for sertindole, Lu 28-092, and Lu 25-073 in human plasma.

The intra-assay accuracy of the method was better for sertindole than for Lu 28-092 and Lu 25-073. Lu 25-073 had the largest within-day deviations from the true mean, possibly due to nonspecific interferences from matrix constituents. The ion channel chosen for Lu 25-073 has a smaller fragment ion (m/z 84) than the other analytes and the molecular weight of the parent ion is also smaller than the other analytes. In the low molecular weight region, there is significantly more noise (higher background chemical noise) than at the higher molecular weights. This high background noise would contribute both to a higher quantitation limit and to larger variability in day to day accuracy. Sertindole also had the best intra-assay precision, averaging approximately 5%. Precision for the two metabolites was not as good as the parent compound; RSDs were approximately 14% for Lu 28-092, and 10% for Lu 25-073. The reason for the larger variation in the assay for Lu 28-092 is thought to be due to the relative instability of the molecule during the sample preparation steps. Sensitivity to laboratory lighting was observed in other in-house studies for this analyte (results not shown).

The pooled mean for all the assays performed over the 6-month period and the associated RSD provided an estimate of the inter-assay accuracy and precision of the method. The accuracy for all three analytes was within 12% of the theoretical concentration for all three concentration levels, indicating that the method has excellent specificity. No distinguishable trends were observed over time, as shown by the control chart for sertindole (Figure 5). Results for Lu 28-092 and Lu 25-073 were similar. Inter-assay precision has a trend similar to intra-assay precision, but is larger in magnitude because this measure also incorporates variation arising from day-to-day implementation of the method (such as: changes in instrument performance, technique differences between operators, effects of multiple freeze/thaw cycles on the sample, etc.). The inter-assay precision for sertindole was again the best, with a RSD of approximately 9% for the three QC concentration levels. The variation for Lu 28-092 was the largest, with a RSD of approximately 19%; as mentioned above, this is suspected to be due to relative instability of the molecule to processing. The inter-assay RSD for Lu 25-073 was approximately 15%.

The inter-assay accuracy and precision for the three analytes indicates that the method is sufficiently robust for GLP compliant production runs. The following criterion was adopted for accepting an analytical batch for GLP-regulated assignments: a QC sample was acceptable if its observed concentration deviated by less than or equal to 20% of the theoretical value. At least two thirds of the measured QCs in the batch were required to be acceptable; otherwise the whole batch and the resulting values for the unknowns were rejected. The expected batch rejection rate based on this criterion, given the accuracy and precision of the method, would be 15% to 20%.

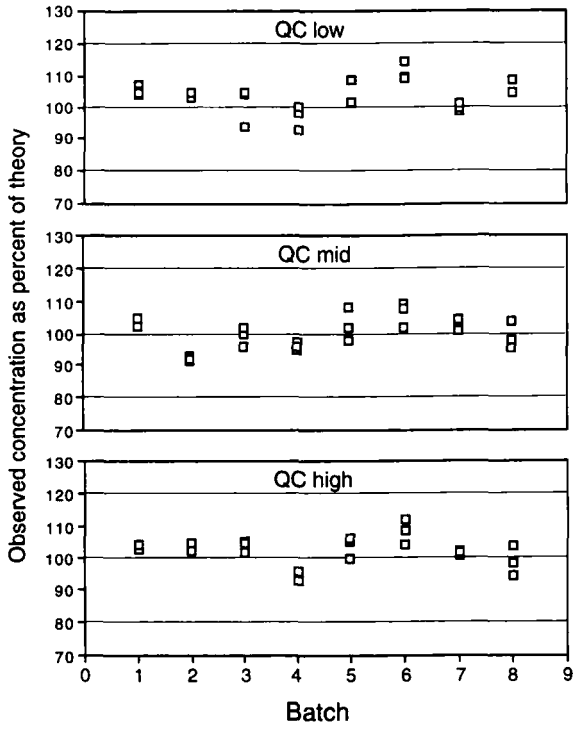

Figure 5. Variation in concentration of sertindole in quality control samples from eight assay batches over a 6 month period. No distinguishable trends were observed over time.

The inter-assay precision and accuracy for sertindole, Lu 28-092, and Lu 25-073 in dog, rat, and mouse plasma are shown in Table 2. The numbers appear to be better than those in human plasma. This may be due to the fact that the experiments in animal plasma were conducted over a shorter time span. Also, the smaller volume extracted for the animal studies would be expected to reduce the effect of matrix on the HPLC column and the ionization interface, such as the buildup of residue and non-eluting compounds on the HPLC column, buildup of non-volatiles on the ionization interface, late eluting peaks that are co-extracted from the matrix, etc. In general, the method was found to perform similarly in the different matrices, and there were no additional issues of stability or interferences that needed to be taken into consideration.

Table 2

Inter-Assay Accuracy and Precision of Assays for Sertindole and Metabolites in Dog, Rat, and Mouse Plasma

	Dog	Rat	Mouse
		Sertindole	
Accuracy*			
QC low	99.8	101.3	104.0
QC mid	100.3	94.7	102.0
QC high	98.4	101.6	100.7
Precision**			
QC low	5.6	7.3	10.0
QC mid	7.0	11.0	6.0
QC high	5.5	6.6	7.2
		Lu 28-092	
Accuracy			
QC low	93.8	106.8	103.8
QC mid	109.6	88.6	104.7
QC high	101.7	98.9	100.9
Precision			
QC low	12.9	6.2	13.2
QC mid	6.7	3.4	12.2
QC high	6.3	10.2	8.8
		Lu 25-073	
Accuracy			
QC low	98.9	104.6	105.3
QC mid	98.7	97.0	104.1
QC high	90.7	98.5	104.0
Precision			
QC low	7.8	10.6	10.5
QC mid	8.8	8.7	11.7
QC high	9.8	11.2	9.0

Note: In dog plasma, the nominal concentrations for low, middle, and high QC levels were 3.4, 16, and 64 ng/mL, respectively. In rat plasma, they were 5.0, 28, and 127 ng/mL respectively. In mouse plasma, they were 6.3, 25, and 127 ng/mL, respectively.

* Mean concentration, expressed as pct. of the theoretical concentration.

** RSDs for replicates were analyzed on different days.

Table 3

Long-Term Frozen Stability of Sertindole and Metabolites

Storage		Sertindole	Lu28-092	Lu 25-073
1 day	High	93.5	94.7	103.6
	Low	103.5	96.2	111.9
6 months	High	88.9	84.9	97.9
	Low	99.4	85.9	98.5
1 year	High	80.4	79.3	101.3
	Low	89.1	75.9	103.1
18 months	High	76.4	76.1	94.6
	Low	84.8	78.3	97.8
2 years	High	75.2	68.2	92.4
	Low	78.0	62.3	95.0

Note: Nominal concentrations for the low and high QC levels were 20 and 100 ng/mL. Observed concentrations were the mean values from five replicate samples at each concentration level.

Long-Term Frozen Stability of Sertindole and Its Metabolites

Because some clinical studies are of long duration and necessitate extended sample storage under frozen conditions, we investigated the stability of the three analytes by storing QC samples prepared in human serum for over a period of 2 years at -20°C. Serum was used for this experiment because some of the earlier clinical trials were conducted using serum as a matrix. Experiments have shown that there is no significant difference in the behavior of the analytes in serum or plasma.[6] The QC samples were aliquoted before storage so that each sample was thawed only once when it was withdrawn for analysis. Representative samples were withdrawn at various time intervals over a two year period and assayed against freshly prepared standards. Samples were assayed in replicates of five to obtain good estimates of the stability over this time period. Two concentration levels were examined: approximately 20 and 100 ng/mL (Table 3). For up to 6 months of frozen storage, all analytes were fairly stable; the decrease was less than 15% of theoretical concentration.

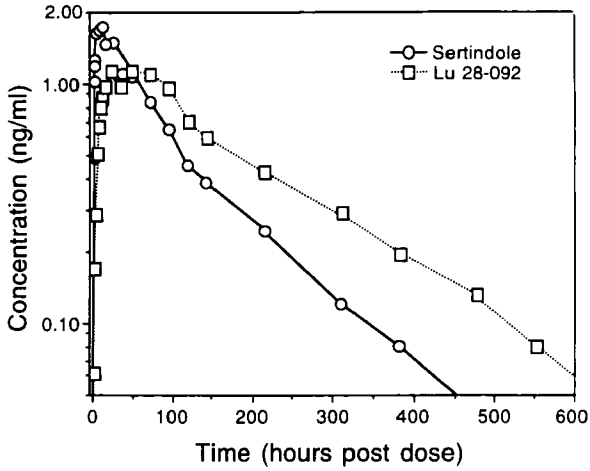

Figure 6. Example of the results obtained in clinical studies using the method. Mean concentration profile of sertindole and Lu 28-092 in a pharmacokinetic study in healthy male subjects.

However, over longer periods, there is an appreciable decrease in the observed concentration of Lu 28-092 and to a smaller extent, sertindole. The Lu 25-073 metabolite seems to be stable and could be stored for extended periods without perceptible loss of analyte.

The assay described above has been used in various clinical and preclinical studies. Figure 6 is an example of the results obtained in such a study. Figure 6 is a plot of the mean concentration profile of sertindole and Lu 28-092 from a clinical study in healthy adult males. Subjects were administered a fairly low oral dose of sertindole (4 mg). The maximal concentration (C_{max}, mean of 16 subjects) was less than 2 ng/mL for both sertindole and Lu 28-092, however, the concentration profiles for both compounds are properly characterized as shown in the figure. The concentrations of Lu 25-073 for the single 4-mg dose were generally below the detection limit of 0.2 ng/mL. Lu 25-073 concentrations have been measured in other studies where subjects are administered higher or multiple doses of sertindole.

CONCLUSION

The HPLC MS/MS assay method for sertindole and its metabolites was found to be sensitive and robust. Results from the human plasma assay were comparable with those from the dog, rat, and mouse plasma assays, indicating that the method is

not significantly affected by compounds co-extracted from the various matrices. The linear dynamic range of the method appears to be smaller (less than three orders of magnitude) relative to other spectroscopic techniques. Correct choice of standard levels and weighting can be used to obtain good accuracy at either the low or high end of the standard curve when working within this limited dynamic range.

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