Abstract
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Application of Dried Plasma Spots, Obtained by Micro-Sampling Device for the Analysis of Rigosertib by LC/MS/MS

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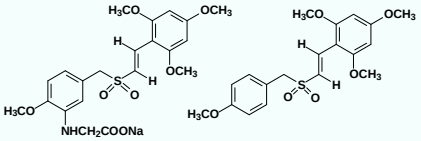
OVERVIEW

- **Purpose** – Develop the method based on dried plasma spots (DPS) obtained by a micro-sampling device to quantify rigosertib in mouse and human plasma using LC/MS/MS.
- **Methods** – DPS were obtained by using (a) filter paper; (b) a micro sampling device, analyte was extracted, and analyzed by LC/MS/MS .
- **Results** – DPS samples, obtained by both filter paper and a micro sampling device, were extracted by several organic solvents and/or solvent/water mixtures. Best results were obtained using methanol/water, 70/30 (v/v) with 0.1 % formic acid. Linearity was $r^2 > 0.999$, over a calibration range of 10 - 40,000 ng/mL for rigosertib, detection limit was 9.6 ng/mL. It was concluded that the DPS, using a micro-sampling device, may be employed as a preferred method in clinical practice. Numerous advantages, including rapid sampling, smaller sample size with fixed volume (~10 µL) than conventional method (40 µL), and capability for safe and convenient sample transport (including air mail) to central laboratories.

BACKGROUND & OBJECTIVE



Rigosertib, ON 01910.Na
$C_{21}H_{24}NNaO_8S$, MW: 473.5 Da

Internal Standard, a Rigosertib derivative,
$C_{19}H_{22}O_6S$, MW: 378.4 Da

- Rigosertib (ON 01910.Na, (E)-{N-[2-methyloxy-5-(2',4',6'-trimethoxy-styryl sulfonyl)methylene phenyl]amino}acetate sodium, MW 473.5 Da), is cytotoxic against a variety of humor tumor cell lines *in vitro* and inhibits growth of tumor xenografts in nude mice (Ref. 1-4).
- DPS techniques, based on filter papers and other paper cards, have been widely used for collecting/storing/shipping plasma samples. A volumetric absorptive micro sampling device was introduced as a novel approach to obtaining a dried plasma sample for determination purpose, which could overcome the sample homogeneity and the area issues associated with conventional DPS when a sub-punch is taken from the sample (Ref. 5-6).
- The objective of this work was to develop the method based on dried plasma spots using the micro sampling device to quantify rigosertib in mouse and human plasma.

METHOD

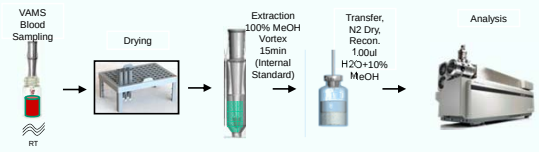


Figure 1. An illustration for the sample preparation using micro-sampling device

Plasma sample collection

- Rigosertib (50 mg/kg) was injected via ip into female mice and blood was collected after 1 h; Patients with advanced cancer were treated with rigosertib (650 – 1,700 mg/m^2), continuous iv for 3 days and each blood sample was collected after 3 h.
- Blood was collected in a tube containing the anticoagulant heparin, then centrifuged at 3,000 rpm (900 *g*) for 10 minutes. Plasma from the top layer was transferred into cryogenic vial and stored in a freezer (-20 ºC or lower) until analyzed.

Sample preparation

- DPS prepared: (a) filter paper - plasma (40 µL) applied to a FTA DMPK C cards (Whatman), (b) micro-sampling device, which holds ~10 µL plasma (Mitra, Phenomenex, CA), was dipped in each sample vials for 5 sec .
- After overnight, DPS were a) filter paper - punched out from the filter papers using a puncher and transferred into vials, b) micro-sampling devices were transferred into vials.
- Internal standard (IS, a rigosertib derivative, 50 mM) were added, analytes were extracted, using sonication for 1 h, with several different solvents including 70/30 methanol/water with 0.1% formic acid. For comparison, equal amounts of plasma (40 and 10 µL) were prepared using protein precipitation with acetonitrile.

LC/MS/MS Conditions

- LC: TSKgel ODS-100V C18 3.5 cm × 2.0 cm i.d., 3 µm (Tosoh Bioscience, PA); flow rate: 0.25 mL/min; Eluent condition – starting with 20% of acetonitrile: water (v/v) with 0.1% formic acid at 5 min after injection, reaching 100 % acetonitrile with 0.1% formic acid at 20 min with initial 5 min cleaning with 100% water using OPTI-LYNX trap cartridge; 10 µL hold C18 0.5 cm x 1.5 mm id (Optimize Technologies, Inc., OR).
- Positive ion electrospray: capillary voltage 3 kV, Cone voltage was 55 V for rigosertib; 15 V for IS. Collision energy was 27 eV for rigosertib; 20 eV for IS.
- The source temperature was 80 ºC, desolvation temperature 250 ºC, nitrogen nebulizer gas flow 80 L/h, desolvation gas flow 800 L/h.

Quantification

- After injecting 10 µL sample aliquots into the ESI source (positive mode), rigosertib concentrations were determined using selected reaction monitoring (SRM) of *m/z* transition from 474 to 216 for rigosertib and from m/z 379 to 120 for the internal standard.
- Quantification was accomplished, with calibration curve, using rigosertib synthetic standard (from 0 ng/mL to 40 µg/mL).

RESULTS

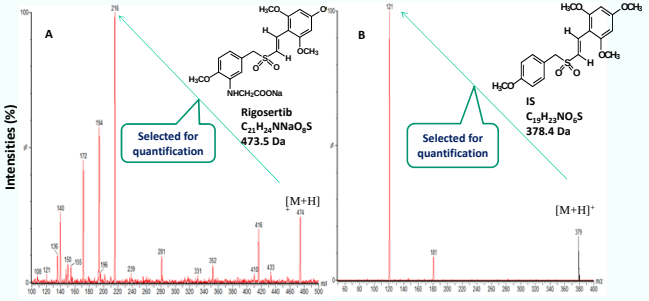


Figure 2. The product ion mass spectra of rigosertib (A) and IS (B).

- In the product ion mass spectra of rigosertib (Figure 2A), m/z 216 is the predominant product ion originating from its molecular ion m/z 474. This reaction was selected for the monitoring of the analyte.
- In the product ion mass spectra of the IS (Figure 2B), m/z 121 is the predominant product ion originating from its molecular ion m/z 379. This reaction was selected for the monitoring of the IS.

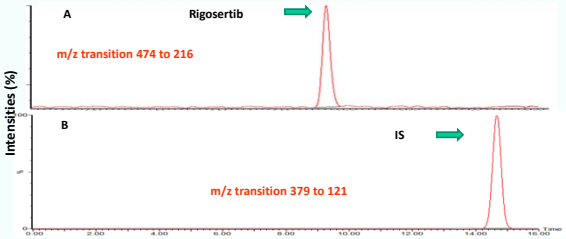


Figure 3. Representative SRM chromatograms of A: rigosertib, B: IS, using a micro-sampling device from a patient plasma sample (1,375 mg/m^2 drug dose).

- DPS samples were obtained both the filter paper and a micro sampling extracted by several organic solvents and solvent/water mixtures; Best results were obtained using methanol/water (v/v) 70/30 with 0.1 % formic acid. Linearity was $r^2 > 0.999$ over a calibration range of 10 ng/mL - 40 µg/mL for rigosertib, detection limit was 9.6 ng/mL.
- Replicate analysis (n=3) of plasma spiked with 500 ng/mL and 5.0 ng/mL yielded RSD of 8.6% and 2.8%, respectively. Intra and inter-day precision and accuracy were < 15%.
- Short term stability of rigosertib in DPS using a micro sampling device, showed no degradation at room temperature (20-25 ºC) when those were kept in foil bags for at least three days.
- Rigosertib amounts were 0.8, 1.2, 1.6, 1.5, 1.6, and 2.3 µg/mL from patients when dose were 650, 1050, 1375, 1375, and 1700 mg/m^2, respectively.
- Rigosertib amount were 1.6 ± 0.2 µg/mL when same dose (50 mg/kg) were repeated on three mice.
- The results were comparable within ± 2%, compared to the conventional technique, based on protein precipitation with acetonitrile.

CONCLUSIONS

- The dried plasma spot technique using a micro-sampling device, may be employed as a preferred method in clinical practice.
- Numerous advantages, including rapid sampling, smaller sample size with fixed volume (~10 µL) than conventional method (40 µL), and capability for safe and convenient sample transport (including air mail) to central laboratories.

REFERENCES and ACKNOWELEDGEMENT

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