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NEW GENERATION Tissue homogenizer
SAMPLE PREPARATION & CELL LYSIS

Precellys
The Science of Lysing

Direct Quantitative Determination of a Nucleotide Diphosphate in Monkey and Rabbit Tissues using LC-MS/MS

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CONTEXT

A sensitive, accurate and precise bioanalytical method was developed for the determination of Cidofovir diphosphate in monkey and rabbit tissues using LC-MS/MS. The method was developed to evaluate concentration levels of the active metabolite in various monkey and rabbit organs and tissues in support of a maximum tolerated dose study [1].

MATERIAL

- Precellys 24.
- Lysing beads were not used to prevent absorption or degradation of the analyte.
- Samples: Monkey and rabbit tissue from liver, lung, spleen, kidney and skin (30 mg each).
- Solvent: 430 µl of 70:30 methanol:water with 30 µl of internal standard.

PROTOCOL

- Precellys 24 parameters: 6500rpm, 1x30 sec.
- The Precellys was used at full capacity (24 samples at a time), followed by centrifugation at 13000 rpm for 5 minutes. The supernatant was filtered through a Sirocco Protein Precipitation plate and transferred to a 96-well collection block for LC-MS/MS analysis.

[1] Foley S, Begley J, Karnik S, Keilholz L, Lambert B. Direct Quantitative Determination of Cidofovir Diphosphate in Monkey and Rabbit Tissues using LC/MS-MS. 2012 ASMS meeting abstracts.

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RESULTS

The Precellys 24 allowed validation of this method over a linear range of 5 to 1000 pg/mg. Full validations were performed for monkey and rabbit liver tissue. Validation experiments included intra- and inter-day precision and accuracy, selectivity/specificity, recovery, matrix effect, lower and upper limit of quantification and stability tests.

Monkey		Accuracy and Precision (mean, CV)		
Tissue	n	Low 15 pg/mg	Med 250 pg/mg	High 750 pg/mg
Liver	24	97.7% (8.2%)	100% (4.8%)	101% (4.3%)
Spleen	6	94.8% (5.4%)	102% (5.1%)	102% (2.6%)
Kidney	6	88.3% (6.0%)	101% (4.2%)	104% (2.3%)
Lung	6	88.1% (6.1%)	93.1% (5.5%)	105% (6.7%)
Skin	6	88.0% (6.7%)	99.2% (3.1%)	101% (3.8%)

Rabbit		Accuracy and Precision (mean, CV)		
Tissue	n	Low 15 pg/mg	Med 250 pg/mg	High 750 pg/mg
Liver	24	102% (7.5%)	101% (4.3%)	102% (3.8%)
Spleen	6	102% (7.4%)	104% (3.8%)	103% (5.7%)
Kidney	6	105% (8.6%)	102% (3.9%)	103% (3.4%)
Lung	6	91.7% (13%)	99.1% (6.9%)	97.8% (4.9%)
Skin	6	106% (6.1%)	98.2% (5.5%)	101% (4.1%)

The accuracy for monkey liver ranged from 97.7% to 101% and the precision ranged from 4.3% to 8.2% (CV). For rabbit liver, the accuracy range was 101% to 102% and the precision range was 4% to 7.5% (CV).

CONCLUSION

A trace quantitative method utilizing the Precellys 24 homogenizer was validated and used for the direct determination of Cidofovir diphosphate in animal studies. Stability tests demonstrated that specific handling techniques were necessary to control degradation and absorption of the analyte during tissue processing and for this reason, beads were not used. **For specific applications in which metabolite absorption is an issue, the Precellys still effectively homogenizes tissue samples due to its high speed and multi-directional motion, even without the use of beads.**

For more details, please contact precellys@bertin.fr

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