Study of capecitabine—An anticancer drug in dried blood spot

Faiyaz Ahmad

Abstract

Capecitabine (Cape), the main oral prodrug which has a place with the gathering of fluoropyrimidine is the most as often as possible recommended anticancer medication for the therapy of metastatic bosom and colorectal malignancies. The strategy was built up over a focus scope of 10-10000 ng/mL. Exactness, accuracy, selectivity, recuperation, lattice impact and soundness of the analyte were likewise assessed and results were inside the acknowledgment rules. Further, exact outcomes were gotten utilizing an ideal spot volume of 10 μL with great spot homogeneity. Blood tests with hematocrit esteems shifting from 24% to 45% gave worthy outcomes with great exactness and accuracy. The productivity of dried blood spot test arrangement, short investigation time and high selectivity grants assessment of Cape in a little blood volume. The approval results recommend that the strategy is exact, precise, and reproducible and can be valuable in restorative medication observing of Cape.

Keywords: Capecitabine, prodrug and fluoropyrimidine

Introduction

The Food and Drug Administration (FDA) had affirmed Cape in 2005 as an oral prodrug of 5-fluorouracil (5-FU) for use as monotherapy in the main line therapy of cutting edge colorectal disease, adjuvant therapy of patients with stage III colon malignancy and privately progressed or metastatic bosom malignancy. In addition, Cape is steadily supplanting 5-FU in a few signs, including gastric malignant growth. The guideline instrument of activity of Cape is the hindrance of thymidylate synthase (TS) and fuse into RNA and DNA. After oral organization and resulting retention over the stomach related parcel, it is changed over to 5-FU through three successive enzymatic responses. It is first processed to 5-deoxy-5-fluorocytidine (5-DFCR) in the liver by the catalyst, carboxylesterase and afterward to 5-deoxy-5-fluorouridine (5-DFUR) in liver and tumor tissue by cytidine deaminase. At last it is changed over intracellularly to 5-FU by thymidine phosphorylase, a protein that is found in tumor tissue. Cape is principally killed as metabolites (> 95% of the portion) and the disposal half-life of the parent medication and its metabolites is around 1.0 h. The bioavailability of Cape is almost 100% and its oral pharmacokinetics is direct, reliant on portion quality. The plasma protein official (predominantly to egg whites) is 54% for Cape and about 10%, 62%, and 10% for its metabolites 5-DFCR, 5-DFUR and 5-FU individually [1-5].

In the most recent decade dried blood spot (DBS) strategy has end up being a prevalent option microsampling approach for quantitative bioanalysis of medications in drug innovative work. The use of DBS and adaption of this approach increased expanding enthusiasm for the drug business as of late. Blood miniature assortment strategy utilizing channel paper has tested the ordinary, obtrusive blood inspecting by venepuncture [6, 7]. DBS method offers particular advantages like decreased example volume assortment (typically 10–25 μL), rearranged test assortment and handling techniques, absence of postcollection preparing, lower expenses of organic example stockpiling and transport, improved (bio)chemical drug strength contrasted and solidified examples, diminished biohazard hazard with least office for capacity and shipment and a lot more [7-9]. Because of such wide scope of preferences it has indicated impressive guarantee for toxicokinetic and pharmacokinetic investigation and become one of the well known microsampling procedures. DBS are likewise regularly utilized in infant and metabolic screening pharmacodynamic contemplates.
irresistible infections the executives, pharmacotoxicological
examines.
The classical approach for the use of DBS samples in
bioanalytical assays is as follows. A small quantity of whole
blood (10–25 μl) is collected from either a fingerprick or
heelprick by a safety lancet or from venous blood onto a
DBS card. The DBS card is then dried at ambient
temperature. From the dried DBS card a fixed area is
punched (completely or partially) and the resulting disk is
subsequently extracted by a solvent prior to analysis (Figure
1). Since the hematocrit (Ht; Percentage of blood cells in
whole blood by volume) has an influence on the spot size
and, thus, the volumetric amount of blood present in the
punched disk, whole cut DBS analysis techniques were
developed to correct for the so-called Ht effect.
A few measures have been accounted for the assurance of
Cape alone [10, 11] alongside its dynamic metabolites in
various natural networks, for example, mouse plasma [12, 13],
mouse serum and bunny bile and human plasma. In two
different reports, Cape has been resolved along with some
multicytostatic mixes. Fundamentally, fluid chromatography
with UV [10, 11, 13, 16] and mass spectrometry
[12, 14, 15, 17] recognition has been utilized for the measurement of Cape
as well as its dynamic metabolites in various grids.

Fig 1: Classical dried blood spot methodology

So as to determine the advantages of DBS, the examinations
must be sufficiently touchy to evaluate the objective analyte
focus in a couple of miniature liters of blood present in a
punched DBS plate. In reality, affectability may here and
there contribute as one of the significant difficulties for DBS
investigation. This issue can be evaded by utilizing delicate
mass spectrometers, for example, triple quadrupole mass
spectrometers through which adequate affectability and
selectivity can be gotten with sufficient certainty. An audit
of writing uncovered no DBS strategies for the quantitation
of Cape utilizing LC-MS/MS and as such bioanalytical
techniques outlining the quantitative examination of
anticancer medications in DBS are exceptionally restricted.
Along these lines, the point of the current investigation was
to create and approve a LC–MS/MS strategy for the
quantitation of Cape in DBS. The strategy was completely
approved dependent on the current administrative rules. The
current strategy gives the effortlessness and accommodation
characteristic to the DBS procedure, quicker run time (~2.5
min) and particularity through MS/MS identification.

Experimental and method
A Shimadzu LC-VP HPLC system (Kyoto, Japan) was used
for chromatographic separation of Cape and IS on
Phenomenex Gemini C18 (150 x 4.6 mm, 5m) column,
maintained at 40 °C in the column oven. The total
chromatographic run time was 2.5 min. For isocratic elution
the mobile phase consisting of acetonitrile and 2mM
ammonium formate (pH 3.0, adjusted with 0.1% formic
acid) in water (80:20, v/v) and was delivered at a flow-rate
of 1.0 mL/min. The total elute from the column was split in
80:20 (v/v) ratio; flow directed to the electrospray interface
was equivalent to 200 μL/min. The autosampler
temperature was maintained at 5 °C and the average pressure of the
system was 1200 psi.
A triple quadrupole mass spectrometer, MDS SCIEX API-4000 (Toronto, Canada), equipped with electro spray ionization and operating in positive ionization mode was
used for detection of analyte and IS. For quantitation,
multiple reaction monitoring (MRM) was used to monitor
precursor → product ion transitions for Cape and IS
respectively.
Sample preparation was performed by spotting 10-μL
fortified blood sample (CS or QC) onto the centre of the
printed circle and left to dry under ambient room
temperature for at least 2 h (Figure 2). A 3-mm diameter
disc was punched (Harris Micro-Punch®, 3-mm circle) out
of the sample collection card and taken in eppendorf tubes,
followed by addition of 150 μL of 0.1N HCl. The sample
was vortexed and sonicated for about 5 min. An aliquot of
50 μL of IS working solution was added and vortexed for
another 1.0 min. Thereafter the analyte and IS were
extracted with 2.5 mL of ethyl acetate by vortex-mixing for
5 min. Samples were centrifuged at 4000 rpm at 10 °C for
10 min. The supernatant was transferred into pre-labeled
tubes and dried at 40 °C. The residue was reconstituted with
100 μL of mobile phase, briefly vortexed and 10 μL was injected into LC-MS/MS system for analysis.

**Results and Discussion**

So as to check ideal reaction for Cape and IS, both positive and negative ionization modes were tried. The sign forces got were a lot higher in the positive mode than in the negative particle mode since Cape and IS can acknowledge protons. This can be credited to the fundamental idea of Cape which has a generally high pKa estimation of 9.5 and subsequently gets promptly protonated under the enhanced acidic portable stage conditions. The full sweep Q1 MS spectra got by imbuing 500 ng/mL arrangements of Cape and IS contained plentiful protonated antecedent particles at m/z 360.1 and 371.3 individually. The most bountiful and trademark item particles were found at m/z 244.4 and 255.1 because of the loss of 2-methyltetrahydrofuran-3,4-diol moiety from the structure of Cape and Cape-d11.

In the current work, boundaries which are DBS explicit like dried blood spot volume, spot spreadability and impact of hematocrit were likewise concentrated as per the EBF suggestions for DBS examination [18]. The acknowledgment rules for these investigations were same as those for dependability appraisals. To test the impact of DBS volume on the exactness and accuracy of the technique, diverse spot volumes (10, 15, 20, 25 and 30 μL) at LQC and HQC levels were investigated in three-fold. It was discovered that at volumes ≥ 20 μL the outcomes were less exact contrasted with lower volumes (10 and 15 μL) as appeared in Table 1. In the current work 10 μL spot volume was chosen dependent on better exactness and accuracy information procured.

**Table 1:** Effect of DBS volumes on the quality control sample concentration (n = 3)

<table>
<thead>
<tr>
<th>QC level</th>
<th>Spot volume (μL)</th>
<th>Concentration found (ng/mL)</th>
<th>Accuracy (%)</th>
<th>Precision (% CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low quality control (30.0 ng/mL)</td>
<td>10</td>
<td>30.4</td>
<td>101.3</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>29.1</td>
<td>97.0</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>28.5</td>
<td>95.1</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>27.9</td>
<td>93.0</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>26.7</td>
<td>89.1</td>
<td>4.2</td>
</tr>
<tr>
<td>High quality control (8000 ng/mL)</td>
<td>10</td>
<td>7946</td>
<td>99.3</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>8262</td>
<td>103.3</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>7643</td>
<td>95.5</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>7427</td>
<td>92.8</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>7144</td>
<td>89.3</td>
<td>2.8</td>
</tr>
</tbody>
</table>

n: Number of replicates; CV: coefficient of variation

The effect of test spreadability was explored by spotting 10 μL of spiked K3EDTA entire blood at LQC and HQC levels in three-fold. The 3 mm plate punched and broke down indicated worthy exactness and accuracy results. The exactness fluctuated from 94.7% to 102.3% and the accuracy (% CV) was in the scope of 1.5-5.2% at the contemplated QC levels.

The impact of hematocrit which characterizes the general volume of red platelets in entire blood enormously impacts the spot homogeneity. QC tests were set up from entire blood having diverse hematocrit levels (24.5%, 33.6% and 44.2%). The spiked examples at LQC and HQC levels (in three duplicates) were readied utilizing these entire blood tests with various hematocrit levels. The exactness and accuracy of the DBS tests went from 92.7-102.3% and ≤ 4.5% individually (Table 2). These outcomes show no effect of hematocrit on the exactness and accuracy of the created technique.

**Table 2:** Impact of hematocrit value on the quality control sample concentration (n = 3)

<table>
<thead>
<tr>
<th>QC level</th>
<th>Hematocrit (%)</th>
<th>Concentration found (ng/mL)</th>
<th>Accuracy (%)</th>
<th>Precision (% CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low quality control (50.0 ng/mL)</td>
<td>24.5</td>
<td>30.7</td>
<td>102.3</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>33.6</td>
<td>28.1</td>
<td>93.6</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>44.2</td>
<td>27.8</td>
<td>92.7</td>
<td>3.6</td>
</tr>
<tr>
<td>High quality control (8000 ng/mL)</td>
<td>24.5</td>
<td>7856</td>
<td>98.2</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>33.6</td>
<td>7806</td>
<td>97.6</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>44.2</td>
<td>7764</td>
<td>97.1</td>
<td>3.8</td>
</tr>
</tbody>
</table>

n: Number of replicates; CV: coefficient of variation

The created strategy is the first endeavor to decide Cape in Quite a while utilizing any known diagnostic procedure. Additionally, it utilizes the least example volume for preparing when contrasted with all past techniques [10, 11, 14]. In spite of the fact that the affectability is less contrasted with a portion of these procedures [15, 17, 18] however the current strategy requires just 2.5 min for chromatographic investigation, which is not exactly these techniques. A near synopsis of the current techniques and the notable highlights of the created DBS strategy.

**Conclusion**

A tough and powerful dried blood spots examine combined with was created and approved for the extraction and examination of Cape in human entire blood. The strategy offers critical points of interest over those recently detailed, regarding lower test necessities, straightforwardness of extraction technique and generally investigation time. The recuperation got was quantitative and predictable across QC levels with no impedance from endo...


